TABLE 3. SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE MYSID, *MYSIDOPSIS BAHIA*, SEVEN DAY SURVIVAL, GROWTH, AND FECUNDITY TEST WITH EFFLUENTS AND RECEIVING WATERS (TEST METHOD 1007.0)[1]

1.	Test type:	Static renewal (required)
2.	Salinity:	20‰ to 30‰ (± 2‰ of the selected test salinity) (recommended)
3.	Temperature:	26 ± 1°C (recommended) Test temperatures must not deviate (i.e., maximum minus minimum temperature) by more than 3°C during the test (required)
4.	Light quality:	Ambient laboratory illumination (recommended)
5.	Light intensity:	10-20 μE/m^2/s (50-100 ft-c.) (ambient laboratory levels) (recommended)
6.	Photoperiod:	16 h light, 8 h darkness, with phase in/out period (recommended)
7.	Test chamber:	8 oz plastic disposable cups, or 400 mL glass beakers (recommended)
8.	Test solution volume:	150 mL per replicate (recommended minimum)
9.	Renewal of test solutions:	Daily (required)
10.	Age of test organisms:	7 days (required)
11.	No. organisms per test chamber:	5 (required minimum)
12.	No. replicate chambers per concentration:	8 (required minimum)
13.	No. larvae per concentration:	40 (required minimum)
14.	Source of food:	Newly hatched *Artemia* nauplii (less than 24 h old)(required)
15.	Feeding regime:	Feed 150 24 h old nauplii per mysid daily, half after test solution renewal and half after 8-12 h (recommended)
16.	Cleaning:	Pipette excess food from cups daily immediately before test solution renewal and feeding (recommended)

[1] For the purposes of reviewing WET test data submitted under NPDES permits, each test condition listed above is identified as required or recommended (see Subsection 10.2 for more information on test review). Additional requirements may be provided in individual permits, such as specifying a given test condition where several options are given in the method.

TABLE 3. SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE MYSID, *MYSIDOPSIS BAHIA*, SEVEN DAY SURVIVAL, GROWTH, AND FECUNDITY TEST WITH EFFLUENTS AND RECEIVING WATERS (TEST METHOD 1007.0) (CONTINUED)

17. Aeration:	None unless DO falls below 4.0 mg/L, then gently aerate in all cups (recommended)
18. Dilution water:	Uncontaminated source of natural seawater, deionized water mixed with hypersaline brine or artificial sea salts (HW MARINEMIX®, FORTY FATHOMS®, GP2 or equivalent) (available options)
19. Test concentrations:	Effluents: 5 and a control (required) Receiving waters: 100% receiving water (or minimum of 5) and a control (recommended)
20. Dilution factor:	Effluents: ≥ 0.5 series (required) Receiving waters: None, or ≥ 0.5 (recommended)
21. Test duration:	7 days (required)
22. Endpoints:	Survival and growth (required); and egg development (recommended)
23. Test acceptability criteria:	80% or greater survival, average dry weight 0.20 mg or greater in controls (required); fecundity may be used if 50% or more of females in controls produce eggs (required if fecundity endpoint used)
24. Sampling requirements:	For on-site tests, samples collected daily and used within 24 h of the time they are removed from the sampling device. For off-site tests, a minimum of three samples (e.g., collected on days one, three, and five) with a maximum holding time of 36 h before first use (see Section 8, Effluent and Receiving Water Sampling, Sample Handling and Sample Preparation for Toxicity Tests, Subsection 8.5.4) (required)
25. Sample volume required:	3 L per day (recommended)

TABLE 4. DATA FOR *MYSIDOPSIS BAHIA* 7-DAY SURVIVAL, GROWTH, AND FECUNDITY TEST[1]

Treatment	Replicate Chamber	Total Mysids	No. Alive	Total Females	Females w/Eggs	Mean Weight
Control	1	5	4	1	1	0.146
	2	5	4	2	2	0.118
	3	5	5	3	2	0.216
	4	5	5	1	1	0.199
	5	5	5	2	2	0.176
	6	5	5	5	4	0.243
	7	5	5	2	2	0.213
	8	5	4	3	3	0.144
50 ppb	1	5	4	2	1	0.154
	2	5	5	3	1	0.193
	3	5	4	3	2	0.190
	4	5	4	0	0	0.190
	5	5	5	5	2	0.256
	6	5	5	2	1	0.191
	7	5	4	4	1	0.122
	8	5	5	3	1	0.177
100 ppb	1	5	3	3	1	0.114
	2	5	5	2	1	0.172
	3	5	5	1	0	0.160
	4	5	5	2	1	0.199
	5	5	5	3	2	0.165
	6	5	3	1	0	0.145
	7	5	4	4	1	0.207
	8	5	4	0	0	0.186
210 ppb	1	5	5	1	0	0.153
	2	5	4	2	0	0.094
	3	5	1	1	0	0.017
	4	5	4	3	0	0.122
	5	5	3	1	0	0.052
	6	5	4	2	0	0.154
	7	5	4	1	0	0.110
	8	5	4	3	0	0.103
450 ppb	1	5	0	0	0	- -
	2	5	1	0	0	0.012
	3	5	0	0	0	- -
	4	5	1	0	0	0.002
	5	5	0	0	0	- -
	6	5	0	0	0	- -
	7	5	0	0	0	- -
	8	5	2	1	0	0.081

[1] Data provided by Lussier, Kuhn and Sewall, Environmental Research Laboratory, U.S. Environmental Protection Agency, Narragansett, RI.

14.13.2 EXAMPLE OF ANALYSIS OF MYSID, *MYSIDOPSIS BAHIA*, SURVIVAL DATA

14.13.2.1 Formal statistical analysis of the survival data is outlined in Figures 9 and 10. The response used in the analysis is the proportion of animals surviving in each test or control chamber. Separate analyses are performed for the estimation of the NOEC and LOEC endpoints and for the estimation of the LC50 endpoint. Concentrations at which there is no survival in any of the test chambers are excluded from statistical analysis of the NOEC and LOEC, but included in the estimation of the LC, EC, and IC endpoints.

14.13.2.2 For the case of equal numbers of replicates across all concentrations and the control, the evaluation of the NOEC and LOEC endpoints is made via a parametric test, Dunnett's Procedure, or a nonparametric test, Steel's Many-one Rank Test, on the arc sine square root transformed data. Underlying assumptions of Dunnett's Procedure, normality and homogeneity of variance, are formally tested. The test for normality is the Shapiro-Wilk's Test, and Bartlett's Test is used to test for homogeneity of variance. If either of these tests fails, the nonparametric test, Steel's Many-one Rank Test, is used to determine the NOEC and LOEC endpoints. If the assumptions of Dunnett's Procedure are met, the endpoints are estimated by the parametric procedure.

14.13.2.3 If unequal numbers of replicates occur among the concentration levels tested, there are parametric and nonparametric alternative analyses. The parametric analysis is a t-test with the Bonferroni adjustment (see Appendix D). The Wilcoxon Rank Sum Test with the Bonferroni adjustment is the nonparametric alternative.

14.13.2.4 Probit Analysis (Finney, 1971; see Appendix H) is used to estimate the concentration that causes a specified percent decrease in survival from the control. In this analysis, the total mortality data from all test replicates at a given concentration are combined. If the data do not fit the Probit model, the Spearman-Karber method, the Trimmed Spearman-Karber method, or the Graphical method may be used (see Appendices I-K).

14.13.2.5 The proportion of survival in each replicate must first be transformed by the arc sine transformation procedure described in Appendix B. The raw and transformed data, means and variances of the transformed observations at each concentration including the control are listed in Table 5. A plot of the survival data is provided in Figure 11.

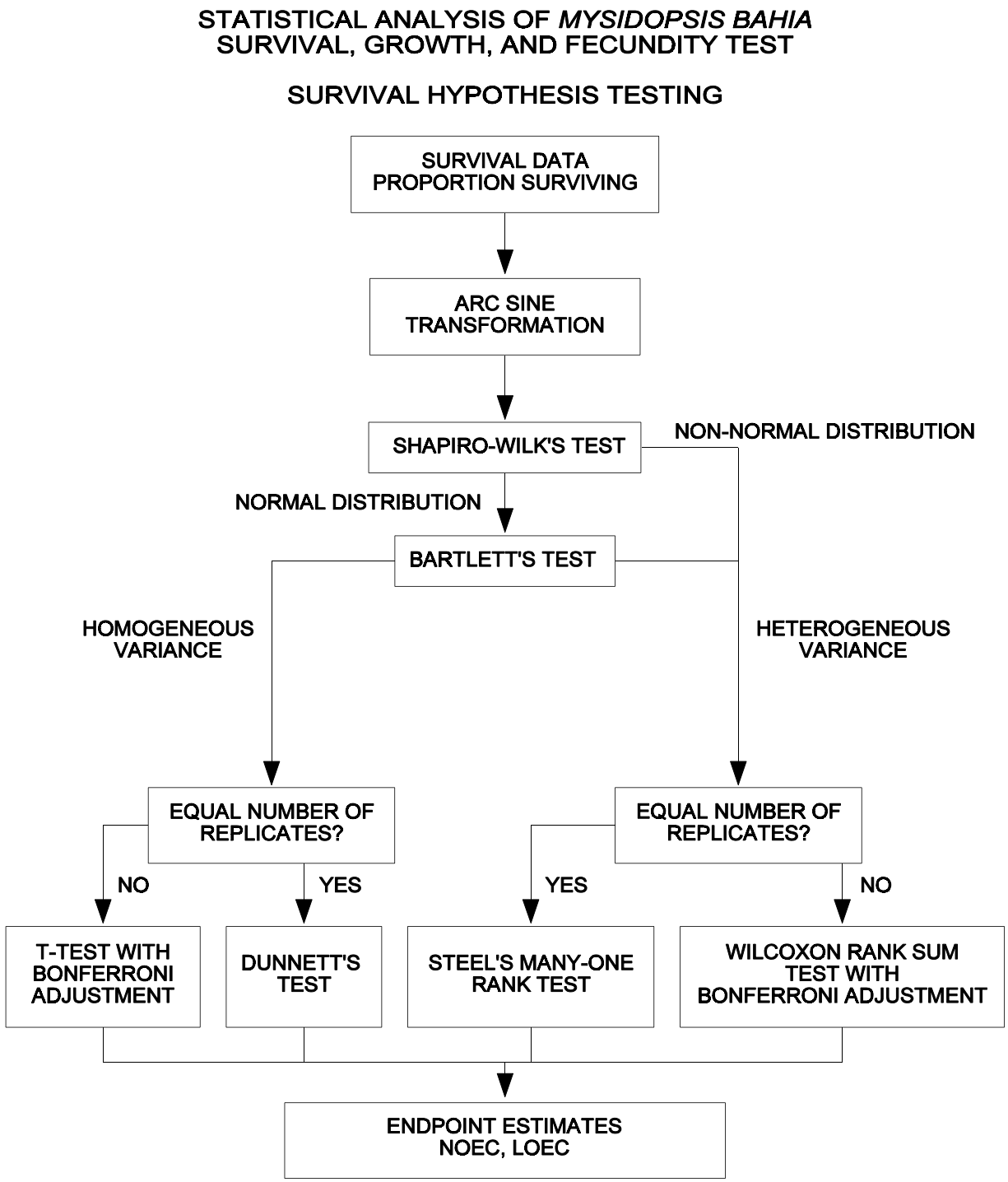

Figure 9. Flowchart for statistical analysis of mysid, *Mysidopsis bahia*, survival data by hypothesis testing.

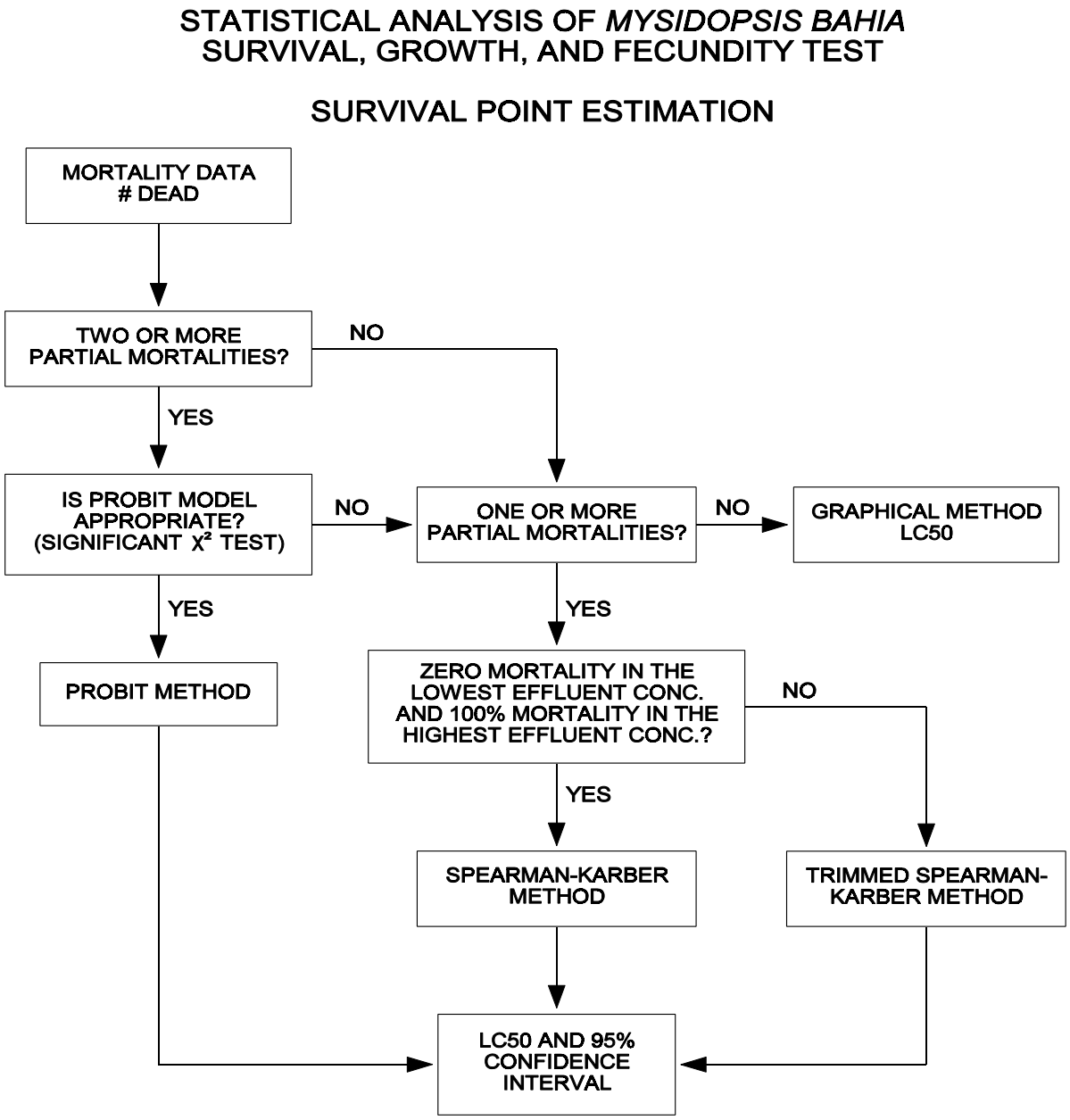

Figure 10. Flowchart for statistical analysis of mysid, *Mysidopsis bahia*, survival data by point estimation.

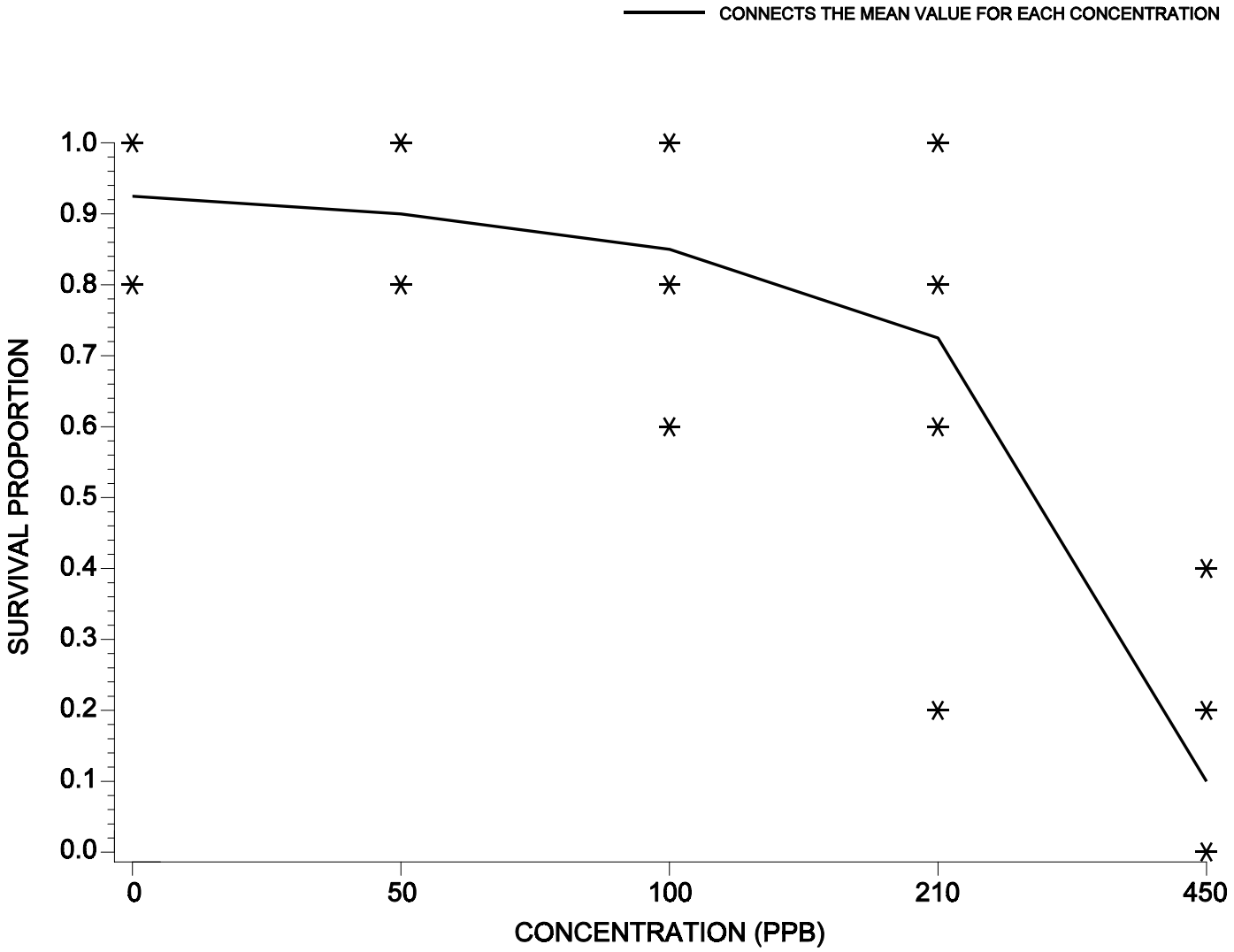

Figure 11. Plot of survival proportions of mysids, *Mysidopsis bahia*, at each treatment level.

TABLE 5. MYSID, *MYSIDOPSIS BAHIA*, SURVIVAL DATA

			Concentration (ppb)			
	Replicate	Control	50.0	100.0	210.0	450.0
RAW	1	0.80	0.80	0.60	1.00	0.00
	2	0.80	1.00	1.00	0.80	0.20
	3	1.00	0.80	1.00	0.20	0.00
	4	1.00	0.80	1.00	0.80	0.20
	5	1.00	1.00	1.00	0.60	0.00
	6	1.00	1.00	0.60	0.80	0.00
	7	1.00	0.80	0.80	0.80	0.00
	8	0.80	1.00	0.80	0.80	0.40
ARC SINE TRANS-FORMED	1	1.107	1.107	0.886	1.345	0.225
	2	1.107	1.345	1.345	1.107	0.464
	3	1.345	1.107	1.345	0.464	0.225
	4	1.345	1.107	1.345	1.107	0.464
	5	1.345	1.345	1.345	0.886	0.225
	6	1.345	1.345	0.886	1.107	0.225
	7	1.345	1.107	1.107	1.107	0.225
	8	1.107	1.345	1.107	1.107	0.685
Mean (Y_i)		1.256	1.226	1.171	1.029	0.342
S_i^2		0.015	0.016	0.042	0.067	0.031
i		1	2	3	4	5

14.13.2.6 Test for Normality

14.13.2.6.1 The first step of the test for normality is to center the observations by subtracting the mean of all observations within a concentration from each observation in that concentration. The centered observations are listed in Table 6.

14.13.2.6.2 Calculate the denominator, D, of the test statistic:

$$D = \sum_{i=1}^{n} (X_i - \bar{X})^2$$

Where: X_i = the ith centered observation

$\bar{X}$ = the overall mean of the centered observations

n = the total number of centered observations.

TABLE 6. CENTERED OBSERVATIONS FOR SHAPIRO-WILK'S EXAMPLE

Replicate	Control (Site Water)	Concentration (ppb) 50.0	100.0	210.0	450.0
1	-0.149	-0.119	-0.285	0.316	-0.117
2	-0.149	0.119	0.174	0.078	0.121
3	0.089	-0.119	0.174	-0.565	-0.117
4	0.089	-0.119	0.174	0.078	0.121
5	0.089	0.119	0.174	-0.142	-0.117
6	0.089	0.119	-0.285	0.078	-0.117
7	0.089	-0.119	-0.064	0.078	-0.117
8	-0.149	0.119	-0.064	0.078	0.342

14.13.2.6.3 For this set of data, $n = 40$

$$\overline{X} = \frac{1}{40}(-0.006) = 0.0$$

$$D = 1.197$$

14.13.2.6.4 Order the centered observations from smallest to largest:

$$X^{(1)} \leq X^{(2)} \leq \ldots \leq X^{(n)}$$

Where $X^{(i)}$ is the ith ordered observation. These ordered observations are listed in Table 7.

14.13.2.6.5 From Table 4, Appendix B, for the number of observations, n, obtain the coefficients $a_1, a_2, \ldots, a_k$ where k is n/2 if n is even and (n-1)/2 if n is odd. For the data in this example, n = 40 and k = 20. The a_i values are listed in Table 8.

14.13.2.6.6 Compute the test statistic, W, as follows:

$$W = \frac{1}{D}\left[\sum_{i=1}^{k} a_i (X^{(n-i+1)} - X^{(i)})\right]^2$$

The differences $X^{(n-i+1)} - X^{(i)}$ are listed in Table 8. For this data in this example:

$$W = \frac{1}{1.197}(1.0475)^2 = 0.9167$$

TABLE 7. ORDERED CENTERED OBSERVATIONS FOR SHAPIRO-WILK'S EXAMPLE

i	$X^{(i)}$	i	$X^{(i)}$
1	-0.565	21	0.078
2	-0.285	22	0.078
3	-0.285	23	0.078
4	-0.149	24	0.089
5	-0.149	25	0.089
6	-0.149	26	0.089
7	-0.143	27	0.089
8	-0.119	28	0.089
9	-0.119	29	0.119
10	-0.119	30	0.119
11	-0.119	31	0.119
12	-0.117	32	0.119
13	-0.117	33	0.121
14	-0.117	34	0.121
15	-0.117	35	0.174
16	-0.117	36	0.174
17	-0.064	37	0.174
18	-0.064	38	0.174
19	0.078	39	0.316
20	0.078	40	0.342

14.13.2.6.7 The decision rule for this test is to compare W as calculated in Subsection 14.13.2.6.6 with the critical value found in Table 6, Appendix B. If the computed W is less than the critical value, conclude that the data are not normally distributed. For this set of data, the critical value at a significance level of 0.01 and n = 40 observations is 0.919. Since W = 0.9167 is less than the critical value, conclude that the data are not normally distributed.

14.13.2.6.8 Since the data do not meet the assumption of normality, Steel's Many-one Rank Test will be used to analyze the survival data.

14.13.2.7 Steel's Many-one Rank Test

14.13.2.7.1 For each control and concentration combination, combine the data and arrange the observations in order of size from smallest to largest. Assign the ranks (1, 2, ... , 16) to the ordered observations with a rank of 1 assigned to the smallest observation, rank of 2 assigned to the next larger observation, etc. If ties occur when ranking, assign the average rank to each tied observation.

14.13.2.7.2 An example of assigning ranks to the combined data for the control and 50.0 ppb concentration is given in Table 9. This ranking procedure is repeated for each control/concentration combination. The complete set of rankings is summarized in Table 10. The ranks are then summed for each concentration level, as shown in Table 11.

TABLE 8. COEFFICIENTS AND DIFFERENCES FOR SHAPIRO-WILK'S EXAMPLE

i	a_i	$X^{(n-i+1)} - X^{(i)}$	
1	0.3964	0.907	$X^{(40)} - X^{(1)}$
2	0.2737	0.601	$X^{(39)} - X^{(2)}$
3	0.2368	0.459	$X^{(38)} - X^{(3)}$
4	0.2098	0.323	$X^{(37)} - X^{(4)}$
5	0.1878	0.323	$X^{(36)} - X^{(5)}$
6	0.1691	0.323	$X^{(35)} - X^{(6)}$
7	0.1526	0.264	$X^{(34)} - X^{(7)}$
8	0.1376	0.240	$X^{(33)} - X^{(8)}$
9	0.1237	0.238	$X^{(32)} - X^{(9)}$
10	0.1108	0.238	$X^{(31)} - X^{(10)}$
11	0.0986	0.238	$X^{(30)} - X^{(11)}$
12	0.0870	0.236	$X^{(29)} - X^{(12)}$
13	0.0759	0.206	$X^{(28)} - X^{(13)}$
14	0.0651	0.206	$X^{(27)} - X^{(14)}$
15	0.0546	0.206	$X^{(26)} - X^{(15)}$
16	0.0444	0.206	$X^{(25)} - X^{(16)}$
17	0.0343	0.153	$X^{(24)} - X^{(17)}$
18	0.0244	0.142	$X^{(23)} - X^{(18)}$
19	0.0146	0.0	$X^{(22)} - X^{(19)}$
20	0.0049	0.0	$X^{(21)} - X^{(20)}$

14.13.2.7.3 For this example, determine if the survival in any of the concentrations is significantly lower than the survival in the control. If this occurs, the rank sum at that concentration would be significantly lower than the rank sum of the control. Thus compare the rank sums for the survival at each of the various concentration levels with some "minimum" or critical rank sum, at or below which the survival would be considered significantly lower than the control. At a significance level of 0.05, the minimum rank sum in a test with four concentrations (excluding the control) and eight replicates is 47 (See Table 5, Appendix E).

14.13.2.7.4 Since the rank sum for the 450 ppb concentration level is less than the critical value, the proportion surviving in that concentration is considered significantly less than that in the control. Since no other rank sums are less than or equal to the critical value, no other concentrations have a significantly lower proportion surviving than the control. Hence, the NOEC and the LOEC are assumed to be 210.0 ppb and 450.0 ppb, respectively.

14.13.2.8 Calculation of the LC50

14.13.2.8.1 The data used for the Probit Analysis is summarized in Table 12. For the Probit Analysis, run the USEPA Probit Analysis Program. An example of the program output is provided in Figure 12.

14.13.2.8.2 For this example, the chi-square test for heterogeneity was not significant. Thus Probit Analysis appears to be appropriate for this set of data.

TABLE 9. ASSIGNING RANKS TO THE CONTROL AND 50 PPB CONCENTRATION LEVEL FOR STEEL'S MANY-ONE RANK TEST

Rank	Transformed Proportion of Total Mortality	Concentration
4	1.107	Control
4	1.107	Control
4	1.107	Control
4	1.107	50 ppb
4	1.107	50 ppb
4	1.107	50 ppb
4	1.107	50 ppb
12	1.571	Control
12	1.571	Control
12	1.571	Control
12	1.571	Control
12	1.571	Control
12	1.571	50 ppb
12	1.571	50 ppb
12	1.571	50 ppb
12	1.571	50 ppb

14.13.3 EXAMPLE OF ANALYSIS OF MYSID, *MYSIDOPSIS BAHIA,* GROWTH DATA

14.13.3.1 Formal statistical analysis of the growth data is outlined in Figure 13. The response used in the statistical analysis is mean weight per original of males and females combined per replicate. Because this measurement is based on the number of original organisms exposed (rather than the number surviving), the measured response is a combined survival and growth endpoint that can be termed biomass. The IC25 and IC50 can be calculated for the growth data via a point estimation technique (see Section 9, Chronic Toxicity Test Endpoints and Data Analysis). Hypothesis testing can be used to obtain an NOEC and LOEC for growth. Concentrations above the NOEC for survival are excluded from the hypothesis test for growth effects.

TABLE 10. TABLE OF RANKS[1]

		Concentration (ppb)			
Replicate	Control	50	100	210	450
1	1.107(4,5,6.5,10)	1.107(4)	0.886(1.5)	1.345(13.5)	0.225(3)
2	1.107(4,5,6.5,10)	1.345(12)	1.345(12)	1.107(6.5)	0.464(6.5)
3	1.345(12,12,13.5,14)	1.107(4)	1.345(12)	0.464(1)	0.225(3)
4	1.345(12,12,13.5,14)	1.107(4)	1.345(12)	1.107(6.5)	0.464(6.5)
5	1.345(12,12,13.5,14)	1.345(12)	1.345(12)	0.886(2)	0.225(3)
6	1.345(12,12,13.5,14)	1.345(12)	0.886(1.5)	1.107(6.5)	0.225(3)
7	1.345(12,12,13.5,14)	1.107(4)	1.107(5)	1.107(6.5)	0.225(3)
8	1.107(4,5,6.5,10)	1.345(12)	1.107(5)	1.107(6.5)	0.685(8)

[1]Control ranks are given in the order of the concentration with which they were ranked.

TABLE 11. RANK SUMS

Concentration	Rank Sum
50	64
100	61
210	49
450	36

14.13.3.2 The statistical analysis using hypothesis tests consists of a parametric test, Dunnett's Procedure, and a nonparametric test, Steel's Many-one Rank Test. The underlying assumptions of the Dunnett's Procedure, normality and homogeneity of variance, are formally tested. The test for normality is the Shapiro-Wilk's Test and Bartlett's Test is used to test for homogeneity of variance. If either of these tests fails, the nonparametric test, Steel's Many-one Rank Test, is used to determine the NOEC and LOEC endpoints. If the assumptions of Dunnett's Procedure are met, the endpoints are determined by the parametric test.

14.13.3.3 Additionally, if unequal numbers of replicates occur among the concentration levels tested, there are parametric and nonparametric alternative analyses. The parametric analysis is a t test with the Bonferroni adjustment. The Wilcoxon Rank Sum Test with the Bonferroni adjustment is the nonparametric alternative. For detailed information on the Bonferroni adjustment, see Appendix D.

Probit Analysis of *Mysidopsis bahia* Survival Data

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Proportion Responding Adjusted for Controls
Control	40	3	0.0750	0.0000
50.0000	40	4	0.1000	-0.0080
100.0000	40	6	0.1500	0.0480
210.0000	40	11	0.2750	0.1880
450.0000	40	36	0.9000	0.8880

Chi - Square for Heterogeneity (calculated) = 0.725
Chi - Square for Heterogeneity (tabular value) = 5.991

Probit Analysis of *Mysidopsis bahia* Survival Data

Estimated LC/EC Values and Confidence Limits

Point		Exposure Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
LC/EC	1.00	123.112	65.283	165.552
LC/EC	50.00	288.873	239.559	335.983

Figure 12. Output for USEPA Probit Analysis Program, Version 1.5.

TABLE 12. DATA FOR PROBIT ANALYSIS

		Concentration (ppb)			
	Control	50.0	100.0	210.0	450.0
No Dead	3	4	6	11	36
No Exposed	40	40	40	40	40

14.13.3.4 The data, mean and variance of the observations at each concentration including the control for this example are listed in Table 13. A plot of the data is provided in Figure 14. Since there is significant mortality in the 450 ppb concentration, its effect on growth is not considered.

TABLE 13. MYSID, *MYSIDOPSIS BAHIA*, GROWTH DATA

		Concentration (ppb)			
Replicate	Control	50.0	100.0	210.0	450.0
1	0.146	0.157	0.114	0.153	-
2	0.118	0.193	0.172	0.071	0.012
3	0.216	0.190	0.160	0.017	-
4	0.199	0.190	0.199	0.112	0.002
5	0.176	0.256	0.165	0.052	-
6	0.243	0.191	0.145	0.154	-
7	0.213	0.122	0.207	0.110	-
8	0.144	0.177	0.186	0.103	0.081
Mean (Y_i)	0.182	0.184	0.168	0.101	-
S_i^2	0.00186	0.00145	0.00091	0.00222	-
i	1	2	3	4	5

14.13.3.5 Test for Normality

14.13.3.5.1 The first step of the test for normality is to center the observations by subtracting the mean of all observations within a concentration from each observation in that concentration. The centered observations are listed in Table 14.

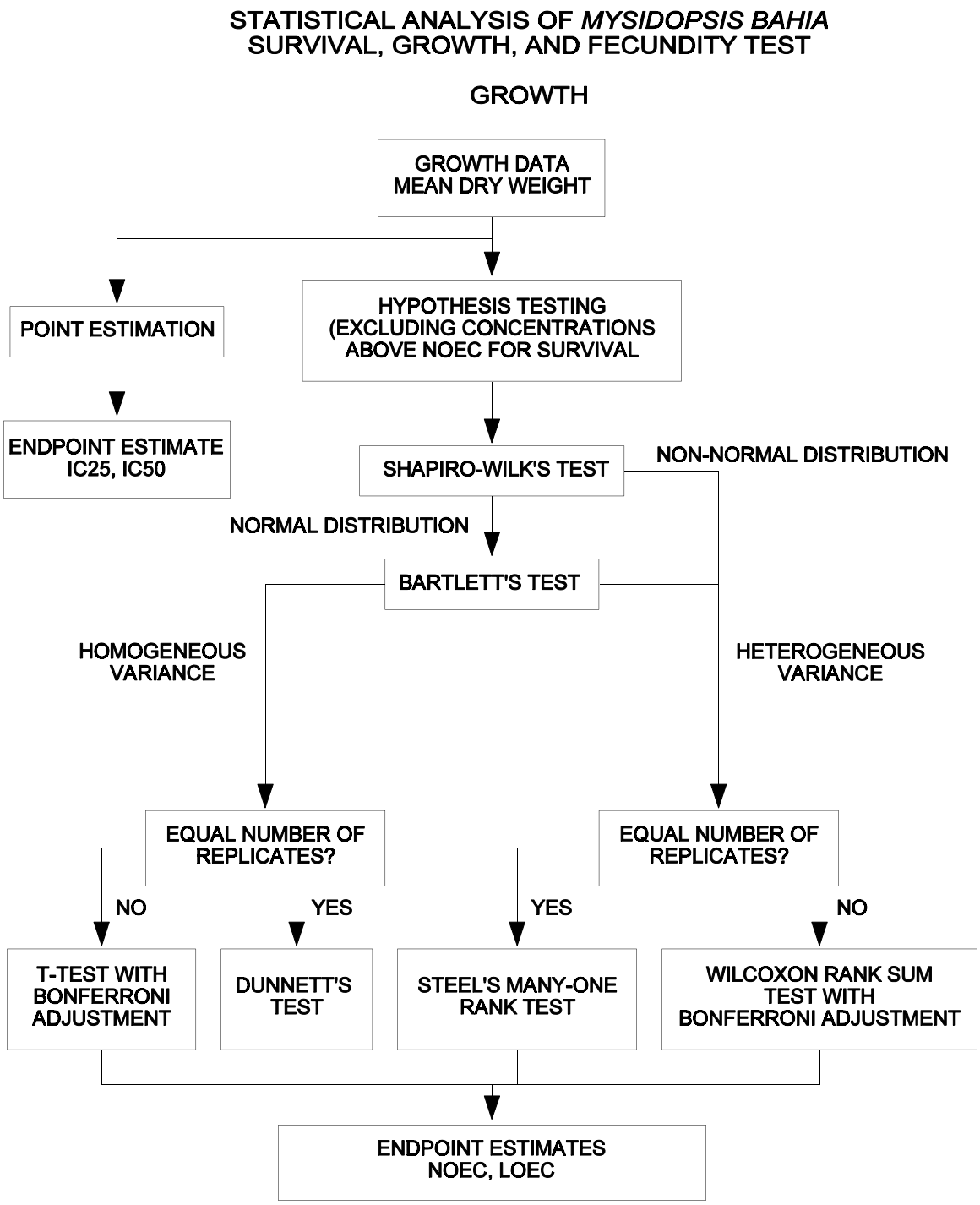

Figure 13. Flowchart for statistical analysis of mysid, *Mysidopsis bahia*, growth data.

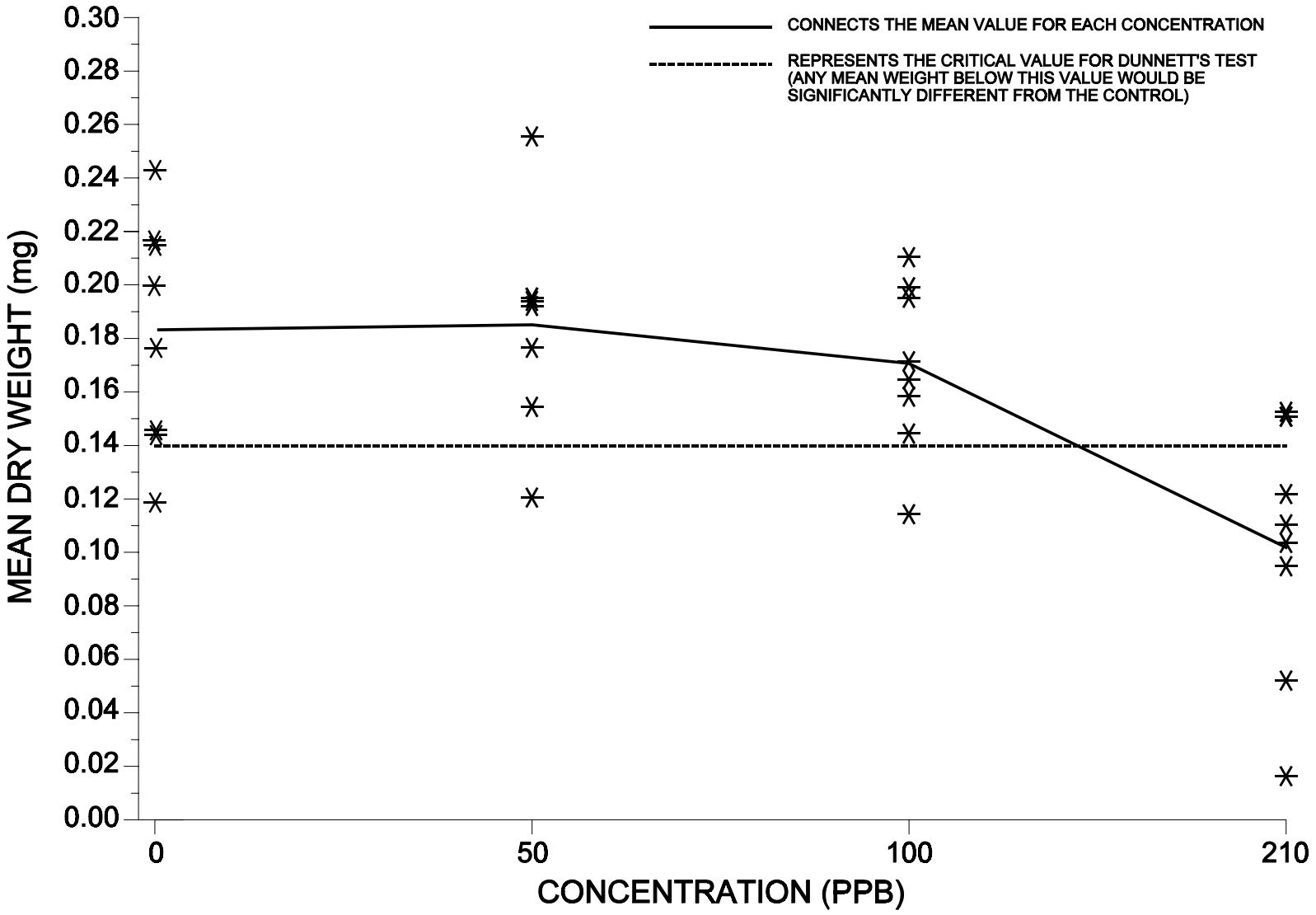

Figure 14. Plot of mean growth data for mysid, *Mysidopsis bahia*, test.

TABLE 14. CENTERED OBSERVATIONS FOR SHAPIRO-WILK'S EXAMPLE

		Concentration (ppb)		
Replicate	Control	50.0	100.0	210.0
1	-0.036	-0.030	-0.054	0.052
2	-0.064	0.009	0.004	-0.007
3	0.034	0.006	-0.008	-0.084
4	0.017	0.006	0.031	0.021
5	-0.006	0.072	-0.003	-0.049
6	0.061	0.007	-0.023	0.053
7	0.031	-0.062	0.039	0.009
8	-0.038	-0.007	0.018	0.002

14.13.3.5.2 Calculate the denominator, D, of the statistic:

$$D = \sum_{i=1}^{n} (X_i - \bar{X})^2$$

Where: X_i = the ith centered observation

$\bar{X}$ = the overall mean of the centered observations

n = the total number of centered observations

14.13.3.5.3 For this set of data, n = 32

$$\bar{X} = \frac{1}{32}(0.007) = 0.000$$

D = 0.0451

14.13.3.5.4 Order the centered observations from smallest to largest

$$X^{(1)} \leq X^{(2)} \leq \ldots \leq X^{(n)}$$

Where $X^{(i)}$ denotes the ith ordered observation. The ordered observations for this example are listed in Table 15.

TABLE 15. ORDERED CENTERED OBSERVATIONS FOR SHAPIRO-WILK'S EXAMPLE

i	$X^{(i)}$	i	$X^{(i)}$
1	-0.084	17	0.006
2	-0.064	18	0.006
3	-0.062	19	0.007
4	-0.054	20	0.009
5	-0.049	21	0.009
6	-0.038	22	0.017
7	-0.036	23	0.018
8	-0.030	24	0.021
9	-0.023	25	0.031
10	-0.008	26	0.031
11	-0.007	27	0.034
12	-0.007	28	0.039
13	-0.006	29	0.052
14	-0.003	30	0.053
15	0.002	31	0.061
16	0.004	32	0.072

14.13.3.5.5 From Table 4, Appendix B, for the number of observations, n, obtain the coefficients a_1, a_2, ... a_k where k is n/2 if n is even and (n-1)/2 if n is odd. For the data in this example, n = 32 and k = 16. The a_i values are listed in Table 16.

14.13.3.5.6 Compute the test statistic, W, as follows:

$$W = \frac{1}{D}[\sum_{i=1}^{k} a_i (x^{(n-i+1)} - x^{(i)}]^2$$

The differences $X^{(n-i+1)} - X^{(i)}$ are listed in Table 16. For this set of data:

$$W = \frac{1}{0.045} (0.2097)^2 = 0.9752$$

14.13.3.5.7 The decision rule for this test is to compare W as calculated in Subsection 14.13.3.5.6 to a critical value found in Table 6, Appendix B. If the computed W is less than the critical value, conclude that the data are not normally distributed. For this set of data, the critical value at a significance level of 0.01 and n = 32 observations is 0.904. Since W = 0.9752 is greater than the critical value, conclude that the data are normally distributed.

TABLE 16. COEFFICIENTS AND DIFFERENCES FOR SHAPIRO-WILK'S EXAMPLE

i	$a_{(i)}$	$X^{(n-i+1)}$	
1	0.4188	0.156	$X^{(32)} - X^{(1)}$
2	0.2898	0.125	$X^{(31)} - X^{(2)}$
3	0.2462	0.115	$X^{(30)} - X^{(3)}$
4	0.2141	0.106	$X^{(29)} - X^{(4)}$
5	0.1878	0.088	$X^{(28)} - X^{(5)}$
6	0.1651	0.072	$X^{(27)} - X^{(6)}$
7	0.1449	0.067	$X^{(26)} - X^{(7)}$
8	0.1265	0.061	$X^{(25)} - X^{(8)}$
9	0.1093	0.044	$X^{(24)} - X^{(9)}$
10	0.0931	0.026	$X^{(23)} - X^{(10)}$
11	0.0777	0.024	$X^{(22)} - X^{(11)}$
12	0.0629	0.016	$X^{(21)} - X^{(12)}$
13	0.0485	0.015	$X^{(20)} - X^{(13)}$
14	0.0344	0.010	$X^{(19)} - X^{(14)}$
15	0.0206	0.004	$X^{(18)} - X^{(15)}$
16	0.0068	0.002	$X^{(17)} - X^{(16)}$

14.13.3.6 Test for Homogeneity of Variance

14.13.3.6.1 The test used to examine whether the variation in mean weight of the mysids is the same across all concentration levels including the control, is Bartlett's Test (Snedecor and Cochran, 1980). The test statistic is as follows:

$$B = \frac{[(\sum_{i=1}^{p} V_i) \ln \bar{S}^2 - \sum_{i=1}^{p} V_i \ln S_i^2]}{C}$$

Where: V_i = degrees of freedom for each copper concentration and control, $V_i = (n_i - 1)$

p = number of concentration levels including the control

ln = $\log_e$

i = 1, 2, ..., p where p is the number of concentrations including the control

n_i = the number of replicates for concentration i.

$$\overline{S}^2 = \frac{(\sum_{i=1}^{P} V_i S_i^2)}{\sum_{i=1}^{P} V_i}$$

$$C = 1 + [3(p-1)]^{-1}[\sum_{i=1}^{p} 1/V_i - (\sum_{i=1}^{p} V_i)^{-1}$$

14.13.3.6.2 For the data in this example (see Table 13), all concentrations including the control have the same number of replicates ($n_i = 8$ for all i). Thus, $V_i = 7$ for all i.

14.13.3.6.3 Bartlett's statistic is therefore:

$$B = [(28)\ln(0.00162) - 7\sum_{i=1}^{p} \ln(S_1^2)]/1.06$$

$$= [28(-6.427) - 7(-25.9329)]/1.06$$

$$= [-179.973 - (-181.530)]/1.06$$

$$= 1.469$$

14.13.3.6.4 B is approximately distributed as chi-square with p - 1 degrees of freedom, when the variances are in fact the same. Therefore, the appropriate critical value for this test, at a significance level of 0.01 with three degrees of freedom, is 11.34. Since B = 1.469 is less than the critical value of 11.34, conclude that the variances are not different.

14.13.3.7 Dunnett's Procedure

14.13.3.7.1 To obtain an estimate of the pooled variance for the Dunnett's Procedure, construct an ANOVA table as described in Table 17.

TABLE 17. ANOVA TABLE

Source	df	Sum of Squares (SS)	Mean Square (MS) (SS/df)
Between	p - 1	SSB	S_B^2 = SSB/(p-1)
Within	N - p	SSW	S_W^2 = SSW/(N-p)
Total	N - 1	SST	

Where: p = number of concentration levels including the control

N = total number of observations $n_1 + n_2 \ldots + n_p$

n_i = number of observations in concentration i

$$SSB = \sum_{i=1}^{p} T_i^2/n_i - G^2/N \qquad \text{Between Sum of Squares}$$

$$SST = \sum_{i=1}^{p} \sum_{j=1}^{n} -j\ Y_{ij}^2 - G^2/N \qquad \text{Total Sum of Squares}$$

$$SSW = SST - SSB \qquad \text{Within Sum of Squares}$$

G = the grand total of all sample observations, $G = \sum_{i=1}^{p} T_i$

T_i = the total of the replicate measurements for concentration i

Y_{ij} = the jth observation for concentration i (represents the mean dry weight of the mysids for concentration i in test chamber j)

14.13.3.7.2 For the data in this example:

$n_1 = n_2 = n_3 = n_4 = 8$

$N = 32$

$T_1 = Y_{11} + Y_{12} + \ldots + Y_{18} = 1.455$

$T_2 = Y_{21} + Y_{22} + \ldots + Y_{28} = 1.473$

$T_3 = Y_{31} + Y_{32} + \ldots + Y_{38} = 1.348$

$T_4 = Y_{41} + Y_{42} + \ldots + Y_{48} = 0.805$

$$G = T_1 + T_2 + T_3 + T_4 = 5.081$$

$$SSB = \sum_{i=1}^{p} T_i^2/n_i - G^2/N$$

$$= \frac{1}{8}(6.752) - \frac{(5.081)^2}{32} = 0.0372$$

$$SST = \sum_{i=1}^{p} \sum_{j=1}^{n_j} Y_{ij}^2 - G^2/N$$

$$= 0.889 - \frac{(5.081)^2}{32} = 0.0822$$

$$SSW = SST - SSB = 0.0822 - 0.0372 = 0.0450$$

$$S_B^2 = SSB / (p-1) = 0.0372 / (4-1) = 0.0124$$

$$S_W^2 = SSW / (N-p) = 0.0450 / (32-4) = 0.0016$$

14.13.3.7.3 Summarize these calculations in the ANOVA table (Table 18).

TABLE 18. ANOVA TABLE FOR DUNNETT'S PROCEDURE EXAMPLE

Source	df	Sum of Squares (SS)	Mean Square(MS) (SS/df)
Between	3	0.0372	0.0127
Within	28	0.0450	0.0016
Total	31	0.0822	

14.13.3.7.4 To perform the individual comparisons, calculate the t statistic for each concentration, and control combination as follows:

$$t_i = \frac{(\bar{Y}_1 - \bar{Y}_i)}{S_w\sqrt{(1/n_1) + (1/n_i)}}$$

Where: $\bar{Y}_i$ = mean dry weight for concentration i

$\bar{Y}_1$ = mean dry weight for the control

S_W = square root of the within mean square

n_1 = number of replicates for the control

n_i = number of replicates for concentration i

14.13.3.7.5 Table 19 includes the calculated t values for each concentration and control combination. In this example, comparing the 50.0 ppb concentration with the control the calculation is as follows:

$$t_2 = \frac{(0.182 - 0.184)}{[0.040\sqrt{(1/8) + (1/8)}]}$$

$$= -0.100$$

TABLE 19. CALCULATED T VALUES

Concentration (ppb)	i	t_i
50.0	2	-0.150
100.0	3	0.700
210.0	4	4.050

14.13.3.7.6 Since the purpose of this test is to detect a significant reduction in mean weight, a one-sided test is appropriate. The critical value for this one-sided test is found in Table 5, Appendix C. For an overall alpha level of 0.05, 28 degrees of freedom for error and three concentrations (excluding the control) the approximate critical value is 2.15. The mean weight for concentration "i" is considered significantly less than the mean weight for the control if t_i is greater than the critical value. Therefore, the 210.0 ppb concentration has significantly lower mean weight than the control. Hence the NOEC and the LOEC for growth are 100.0 ppb and 210.0 ppb, respectively.

14.13.3.7.7 To quantify the sensitivity of the test, the minimum significant difference (MSD) that can be detected statistically may be calculated.

$$MSD = d\ S_w\sqrt{(1/n_1) + (1/n)}$$

Where: d = the critical value for Dunnett's Procedure

S_W = the square root of the within mean square

n = the common number of replicates at each concentration
(this assumes equal replication at each concentration)

n_1 = the number of replicates in the control.

14.13.3.7.8 In this example:

$$MSD = 2.15(0.04)\sqrt{(1/8) + (1/8)}$$

$$= 2.15\ (0.04)(0.5)$$

$$= 0.043$$

14.13.3.7.9 Therefore, for this set of data, the minimum difference that can be detected as statistically significant is 0.043 mg.

14.13.3.7.10 This represents a 23.6% reduction in mean weight from the control.

14.13.3.8 Calculation of the ICp

14.13.3.8.1 The growth data from Table 13 are utilized in this example. As seen in, the observed means are not monotonically non-increasing with respect to concentration. Therefore, it is necessary to smooth the means prior to calculating the ICp. In the following discussion, the observed means are represented by $\bar{Y}_i$ and the smoothed means by M_i.

14.13.3.8.2 Starting with the control mean, $\bar{Y}_1 = 0.182$ and $\bar{Y}_2 = 0.184$, we see that $\bar{Y}_1 < \bar{Y}_2$. Calculate the smoothed means:

$$M_1 = M_2 = (\bar{Y}_1 + \bar{Y}_2)/2 = 0.183$$

14.13.3.8.3 Since $\bar{Y}_5 = 0.025 < \bar{Y}_4 = 0.101 < \bar{Y}_3 = 0.168 < M_2$, set $M_3 = 0.168$ and $M_4 = 0.101$, and $M_5 = 0.025$. Table 20 contains the smoothed means and Figure 15 gives a plot of the smoothed response curve.

TABLE 20. MYSID, *MYSIDOPSIS BAHIA*, MEAN GROWTH RESPONSE AFTER SMOOTHING

Toxicant Conc. (ppb)	i	Response Means Y_i (mg)	Smoothed Mean M_i (mg)
Control	1	0.182	0.183
50.0	2	0.184	0.183
100.0	3	0.168	0.168
210.0	4	0.101	0.101
450.0	5	0.012	0.012

14.13.3.8.4 An IC25 and IC50 can be estimated using the Linear Interpolation Method. A 25% reduction in weight, compared to the controls, would result in a mean weight of 0.136 mg, where $M_1(1\text{-}p/100) = 0.183(1\text{-}25/100)$. A 50% reduction in mean dry weight, compared to the controls, would result in a mean weight of 0.091 mg. Examining the smoothed means and their associated concentrations (Table 20), the response, 0.136 mg, is bracketed by $C_3 = 100$ ppb and $C_4 = 210$ ppb. The response, 0.091 mg, is bracketed by $C_4 = 210$ ppb and $C_5 = 450$ ppb.

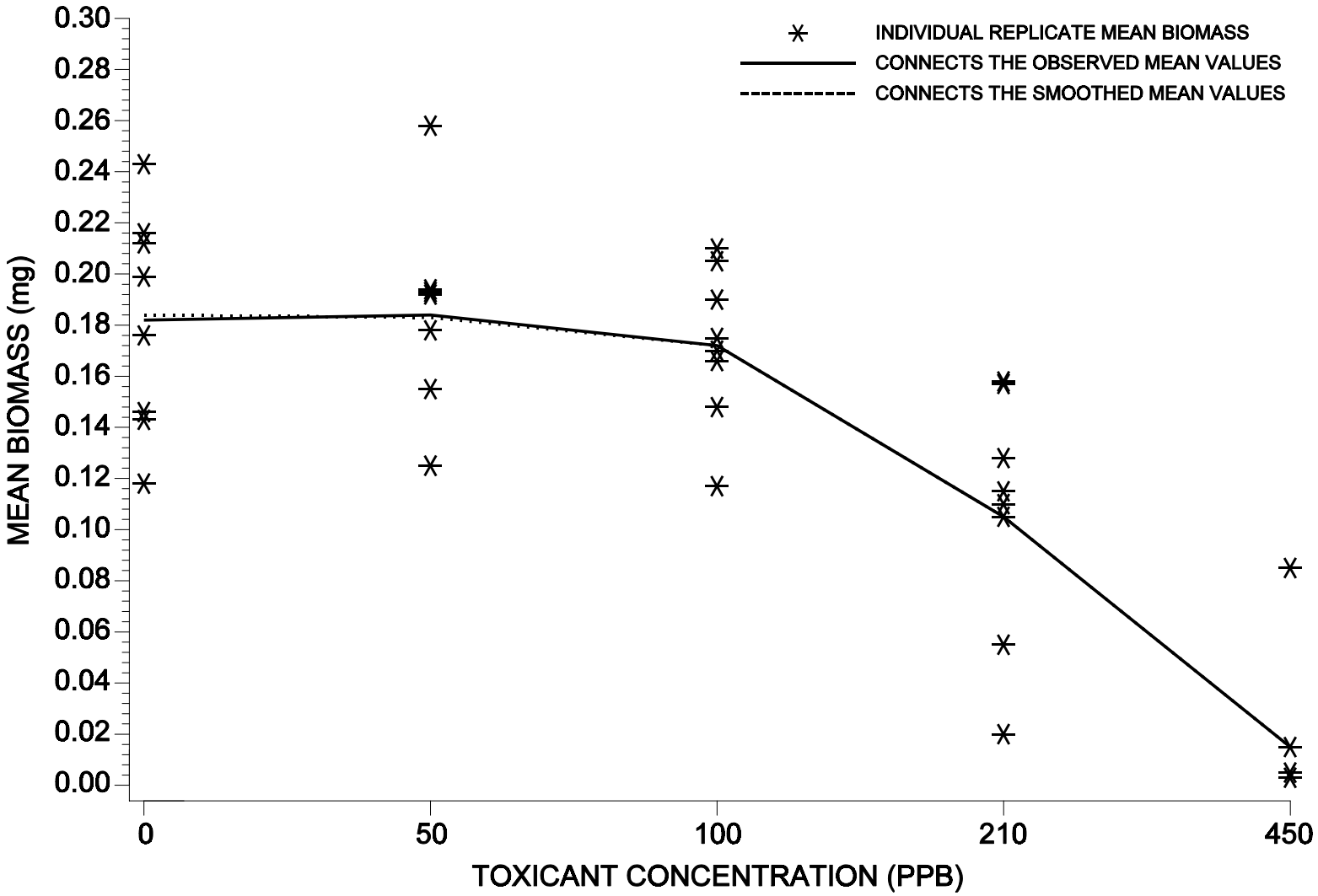

Figure 15. Plot of raw data, observed means, and smoothed means for the mysid, *Mysidopsis bahia*, growth data from Tables 13 and 20.

14.13.3.8.5 Using the equation in Section 4.2 from Appendix L, the estimate of the IC25 is calculated as follows:

$$ICp = C_j + [M_1(1 - p/100) - M_j]\frac{(C_{(j+1)} - C_j)}{M_{(j+1)} - M_j}$$

$$\text{IC25} = 100 + [0.183(1 - 25/100) - 0.168]\ \frac{(210 - 100)}{(0.101 - 0.168)}$$

$$= 151 \text{ ppb.}$$

14.13.3.8.6 Using Equation 1 from Appendix L, the estimate of the IC50 is calculated as follows:

$$ICp = C_j + [M_1(1 - p/100) - M_j]\frac{(C_{(j+1)} - C_j)}{M_{(j=1)} - M_j}$$

$$\text{IC50} = 210 + [0.183(1 - 50/100) - 0.101]\ \frac{(450 - 210)}{(0.012 - 0.101)}$$

$$= 236 \text{ ppb.}$$

14.13.3.8.7 When the ICPIN program was used to analyze this set of data, requesting 80 resamples, the estimate of the IC25 was 150.6446 ppb. The empirical 95.0% confidence interval for the true mean was 97.0905 ppb and 186.6383 ppb. The computer program output for the IC25 for this data set is shown in Figure 16.

14.13.3.8.8 When the ICPIN program was used to analyze this set of data for the IC50, requesting 80 resamples, the estimate of the IC50 was 234.6761 ppb. The empirical 95.0% confidence interval for the true mean was (183.8187 ppb to 277.9211 ppb). The computer program output for the IC50 for this data set is shown in Figure 17.

14.13.4 EXAMPLE OF ANALYSIS OF MYSID, *MYSIDOPSIS BAHIA*, FECUNDITY DATA

14.13.4.1 Formal statistical analysis of the fecundity data is outlined in Figure 18. The response used in the statistical analysis is the proportion of females with eggs in each test or control chamber. If no females were present in a replicate, a response of zero should not be used. Instead there are no data available for that replicate and the number of replicates for that level of concentration or the control should be reduced by one. Separate analyses are performed for the estimation of the NOEC and LOEC endpoints, and for the estimation of the EC, LC, and IC endpoints. The data for a concentration are excluded from the statistical analysis of the NOEC and LOEC endpoints if no eggs were produced in all of the replicates in which females existed. However, all data are included in the estimation of the IC25 and IC50.

Conc. ID	1	2	3	4.	5
Conc. Tested	0	50	100	210	450
Response 1	.146	.154	.114	.153	0
Response 2	.118	.19	.172	.094	.012
Response 3	.216	.193	.160	.017	0
Response 4	.199	.190	.199	.122	.002
Response 5	.176	.190	.165	.052	0
Response 6	.243	.191	.145	.154	0
Response 7	.213	.122	.207	.110	0
Response 8	.144	.177	.186	.103	.081

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Effluent
Test Start Date: Test Ending Date:
Test Species: MYSID SHRIMP, Mysidopsis bahia
Test Duration: growth test
DATA FILE: mysidwt.icp
OUTPUT FILE: mysid.i25

Conc. ID	Number Replicates	Concentration µg/l	Response Means	Standard. Dev.	Pooled Response Means
1	8	0.000	0.182	0.043	0.183
2	8	50.000	0.184	0.038	0.183
3	8	100.000	0.168	0.030	0.168
4	8	210.000	0.101	0.047	0.101
5	8	450.000	0.102	0.028	0.012

The Linear Interpolation Estimate: 150.6446 Entered P Value: 25

Number of Resamplings: 80
The Bootstrap Estimates Mean: 147.1702 Standard Deviation: 23.7984
Original Confidence Limits: Lower: 97.0905 Upper: 186.6383
Resampling time in Seconds: 0.11 Random Seed: -1623038650

Figure 16. ICPIN program output for the IC25.

Conc. ID	1	2	3	4.	5
Conc. Tested	0	50	100	210	450
Response 1	.146	.154	.114	.153	0
Response 2	.118	.193	.172	.094	.012
Response 3	.216	.190	.160	.017	0
Response 4	.199	.190	.199	.122	.002
Response 5	.176	.256	.165	.052	0
Response 6	.243	.191	.145	.154	0
Response 7	.213	.122	.207	.110	0
Response 8	.144	.177	.186	.103	.081

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent:
Test Start Date: Test Ending Date:
Test Species: MYSID SHRIMP, Mysidopsis bahia
Test Duration: growth test
DATA FILE: mysidwt.icp
OUTPUT FILE: mysidwt.i50

Conc. ID	Number Replicates	Concentration μg/L	Response Means	Standard. Dev. Response Means	Pooled
1	8	0.000	0.182	0.043	0.183
2	8	50.000	0.184	0.038	0.183
3	8	100.000	0.168	0.030	0.168
4	8	210.000	0.101	0.047	0.101
5	8	450.000	0.012	0.028	0.01

The Linear Interpolation Estimate: 234.6761 Entered P Value: 50

Number of Resamplings: 80
The Bootstrap Estimates Mean: 230.7551 Standard Deviation: 30.6781
Original Confidence Limits: Lower: 183.8197 Upper: 277.9211
Resampling time in Seconds: 0.16 Random Seed: -628896314

Figure 17. ICPIN program output for the IC50.

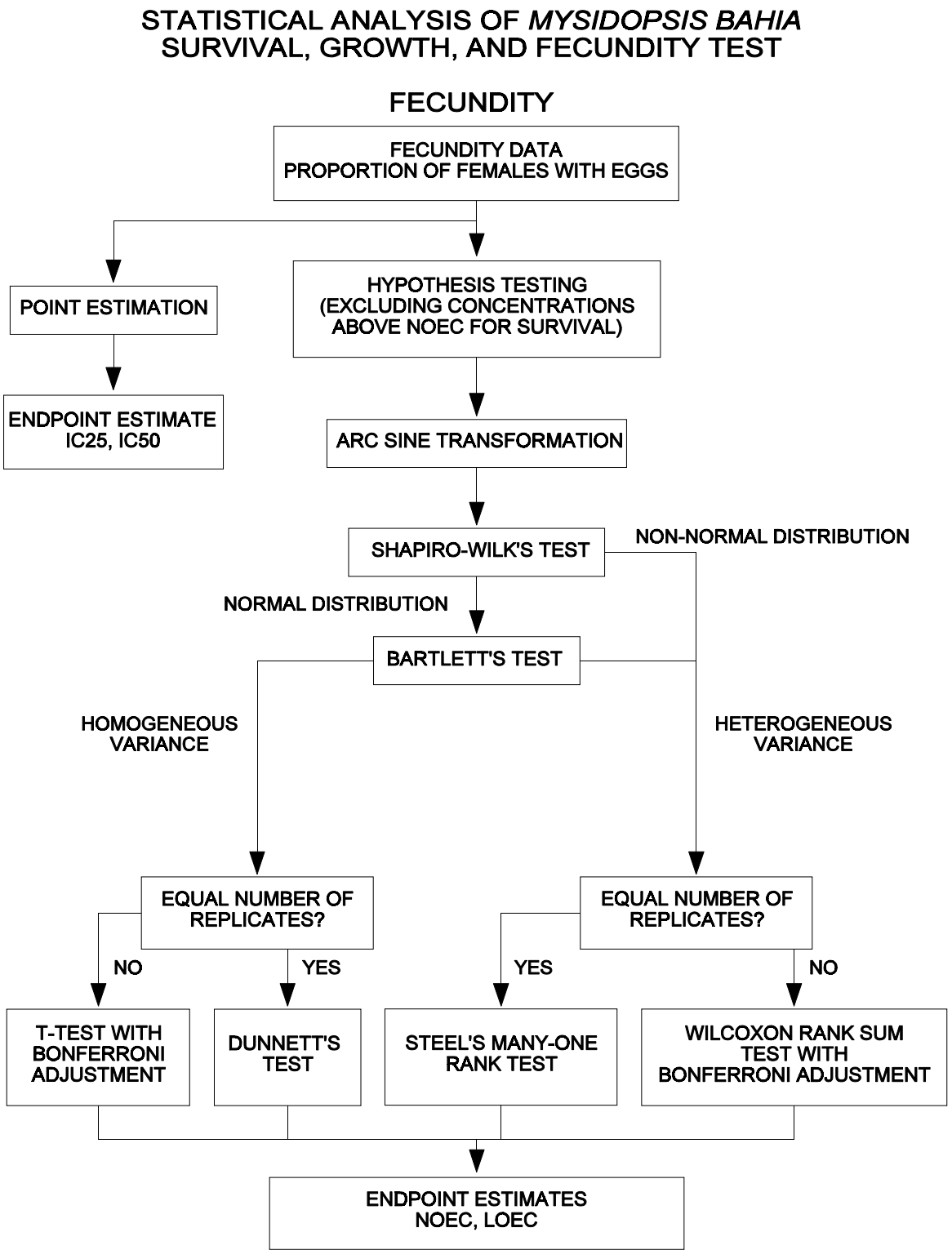

Figure 18. Flowchart for statistical analysis of mysid, *Mysidopsis bahia*, fecundity data.

14.13.4.2 For the case of equal numbers of replicates across all concentrations and the control, the evaluation of the NOEC and LOEC endpoints is made via a parametric test, Dunnett's Procedure, or a nonparametric test, Steel's Many-one Rank Test, on the arc sine square root transformed data. Underlying assumptions of Dunnett's Procedure, normality and homogeneity of variance, are formally tested. The test for normality is the Shapiro-Wilk's Test, and Bartlett's Test is used to test for homogeneity of variance. If either of these tests fails, the nonparametric test, Steel's Many-one Rank Test, is used to determine the NOEC and LOEC endpoints. If the assumptions of Dunnett's Procedure are met, the endpoints are estimated by the parametric procedure.

14.13.4.3 If unequal numbers of replicates occur among the concentration levels tested, there are parametric and nonparametric alternative analyses. The parametric analysis is a t test with the Bonferroni adjustment (Appendix D). The Wilcoxon Rank Sum Test with the Bonferroni adjustment is the nonparametric alternative.

14.13.4.4 The proportion of female mysids, *Mysidopsis bahia*, with eggs in each replicate must first be transformed by the arc sine square root transformation procedure described in Appendix B. Since the denominator of the proportion of females with eggs varies with the number of females occurring in that replicate, the adjustment of the arc sine square root transformation for 0% and 100% is not used for this data. The raw and transformed data, means and variances of the transformed observations at each test concentration including the control are listed in Table 21. Since there is significant mortality in the 450 ppb concentration, its effect on reproduction is not considered. Additionally, since no eggs were produced by females in any of the replicates for the 210 ppb concentration, it is not included in this statistical analysis and is considered a qualitative reproductive effect. A plot of the mean proportion of female mysids with eggs is illustrated in Figure 19.

14.13.4.5 Test for Normality

14.13.4.5.1 The first step of the test for normality is to center the observations by subtracting the mean of all observations within a concentration from each observation in that concentration. The centered observations are listed in Table 22.

14.13.4.5.2 Calculate the denominator, D, of the statistic:

$$D = \sum_{i=1}^{n} (X_i - \bar{X})^2$$

Where: X_i = the ith centered observation

$\bar{X}$ = the overall mean of the centered observations

n = the total number of centered observations

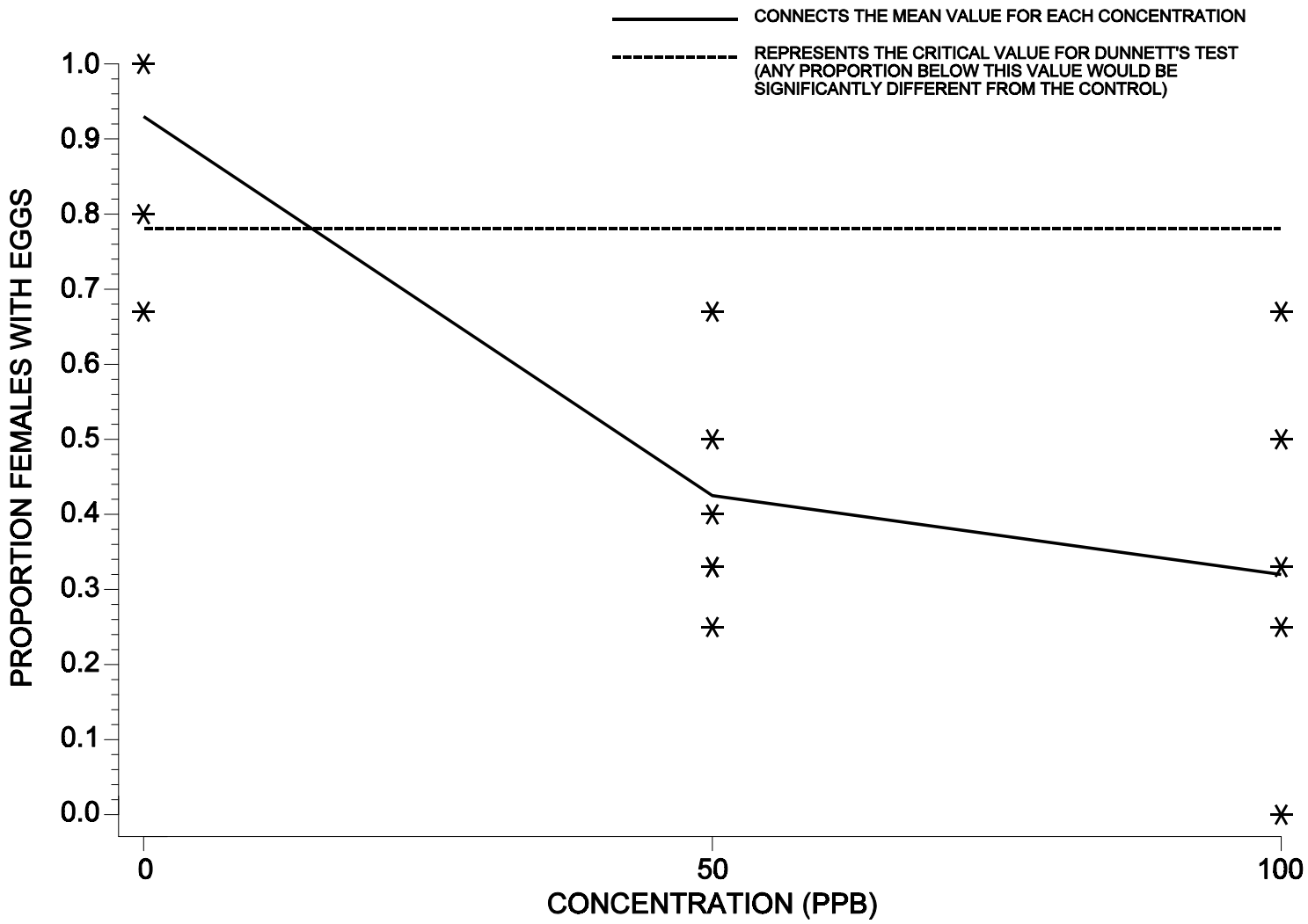

Figure 19. Proportion of female mysids, *Mysidopsis bahia*, with eggs.

TABLE 21. MYSID, *MYSIDOPSIS BAHIA*, FECUNDITY DATA: PERCENT FEMALES WITH EGGS

		Test Concentration (ppb)			
	Replicate	Control	50.0	100.0	210.0
RAW	1	1.00	0.50	0.33	0.0
	2	1.00	0.33	0.50	0.0
	3	0.67	0.67	0.00	0.0
	4	1.00	-	0.50	0.0
	5	1.00	0.40	0.67	0.0
	6	0.80	0.50	0.00	0.0
	7	1.00	0.25	0.25	0.0
	8	1.00	0.33	-	0.0
ARC SINE TRANS-FORMED[1]	1	1.57	0.78	0.61	-
	2	1.57	0.61	0.78	-
	3	0.96	0.96	0.00	-
	4	1.57	-	0.78	-
	5	1.57	0.68	0.96	-
	6	1.12	0.78	0.00	-
	7	1.57	0.52	0.52	-
	8	1.57	0.61	-	-
Mean(Y_i)		1.44	0.71	0.52	-
S^2_i		0.064	0.021	0.147	-
i		1	2	3	4

[1] Since the denominator of the proportion of females with eggs varies with the number of females occurring in that replicate, the adjustment of the arc sine square root transformation for 0% and 100% is not used for this data.

TABLE 22. CENTERED OBSERVATIONS FOR SHAPIRO-WILK'S EXAMPLE

		Test Concentration (ppb)	
Replicate	Control	50.0	100.0
1	0.13	0.07	0.09
2	0.13	-0.10	0.26
3	-0.48	0.25	-0.52
4	0.13	-	0.26
5	0.13	-0.03	0.44
6	-0.32	0.07	-0.52
7	0.13	-0.19	0.00
8	0.13	-0.10	-

14.13.4.5.3 For this set of data, n = 22

$$X = \frac{1}{22}(0.000) = 0.000$$

$$D = 1.4412$$

14.13.4.5.4 Order the centered observations from smallest to largest:

$$X^{(1)} \leq X^{(2)} \leq \ldots \leq X^{(n)}$$

Where $X^{(i)}$ denotes the ith ordered observation. The ordered observations for this example are listed in Table 23.

14.13.4.5.5. From Table 4, Appendix B, for the number of observations, n, obtain the coefficients $a_1, a_2, \ldots a_k$ where k is n/2 if n is even and (n-1)/2 if n is odd. For the data in this example, n = 22 and k = 11. The a_i values are listed in Table 24.

14.13.4.5.6 Compute the test statistic, W, as follows:

$$W = \frac{1}{D}\left[\sum_{i=1}^{k} a_i (X^{(n-i+1)} - X^{(i)})\right]^2$$

The differences $X^{(n-i+1)} - X^{(I)}$ are listed in Table 24. For the data in this example:

$$W = \frac{1}{1.4412}(1.1389)^2 = 0.900$$

TABLE 23. ORDERED CENTERED OBSERVATIONS FOR SHAPIRO-WILK'S EXAMPLE

i	$X^{(i)}$	i	$X^{(i)}$
1	-0.52	12	0.09
2	-0.52	13	0.13
3	-0.48	14	0.13
4	-0.32	15	0.13
5	-0.19	16	0.13
6	-0.10	17	0.13
7	-0.10	18	0.13
8	0.03	19	0.25
9	0.00	20	0.26
10	0.07	21	0.26
11	0.07	22	0.44

TABLE 24. COEFFICIENTS AND DIFFERENCES FOR SHAPIRO-WILK'S EXAMPLE

	i	a_i	$X^{(n-i+1)} - X^{(i)}$
1	0.4590	0.96	$X^{(22)} - X^{(1)}$
2	0.3156	0.78	$X^{(21)} - X^{(2)}$
3	0.2571	0.74	$X^{(20)} - X^{(3)}$
4	0.2131	0.57	$X^{(19)} - X^{(4)}$
5	0.1764	0.32	$X^{(18)} - X^{(5)}$
6	0.1443	0.23	$X^{(17)} - X^{(6)}$
7	0.1150	0.23	$X^{(16)} - X^{(7)}$
8	0.0878	0.16	$X^{(15)} - X^{(8)}$
9	0.0618	0.13	$X^{(14)} - X^{(9)}$
10	0.0368	0.06	$X^{(13)} - X^{(10)}$
11	0.0122	0.02	$X^{(12)} - X^{(11)}$

14.13.4.5.7 The decision rule for this test is to compare W as calculated in Subsection 14.13.4.5.6 to a critical value found in Table 6, Appendix B. If the computed W is less than the critical value, conclude that the data are not normally distributed. For this set of data, the critical value at a significance level of 0.01 and n = 22 observations is 0.878. Since W = 0.900 is greater than the critical value, conclude that the data are normally distributed.

14.13.4.6 Test for Homogeneity of Variance

14.13.4.6.1 The test used to examine whether the variation in proportion of female mysids with eggs is the same across all concentration levels including the control, is Bartlett's Test (Snedecor and Cochran, 1980). The test statistic is as follows:

$$B = \frac{[(\sum_{i=1}^{p} V_i) \ln \overline{S}^2 - \sum_{i=1}^{p} V_i \ln S_i^2]}{C}$$

Where: V_i = degrees of freedom for each copper concentration and control, $V_i = (n_i - 1)$

p = number of concentration levels including the control

$$\overline{S}^2 = \frac{(\sum_{i=1}^{p} V_i S_i^2)}{\sum_{i=1}^{p} V_i}$$

ln = $\log_e$

i = 1, 2, ..., p where p is the number of concentrations including the control

n_i = the number of replicates for concentration i.

$$C = 1+[3(p-1)^{-1}\ [\sum_{i=1}^{p} 1/V_i - (\sum_{i=1}^{p} V_i)^{-1}]$$

14.13.4.6.2 For the data in this example (see Table 21), $n_1 = 8$, $n_2 = 7$ and $n_3 = 7$. Thus, the respective degrees of freedom are 7, 6 and 6.

14.13.4.6.3 Bartlett's statistic is therefore:

$$B = [(19)\ln(0.077) - (7\ln(0.064) + 6\ln(0.021) + 6\ln(0.147))]/1.07$$

$$= [19(-2.564) - (-53.925)]/1.07$$

$$= [-48.716 - (-53.925)]/1.07$$

$$= 4.868$$

14.13.4.6.4 B is approximately distributed as chi-square with p - 1 degrees of freedom, when the variances are in fact the same. Therefore, the appropriate critical value for this test, at a significance level of 0.01 with two degrees of freedom, is 9.210. Since B = 4.868 is less than the critical value of 9.210, conclude that the variances are not different.

14.13.4.7 T test with the Bonferroni Adjustment

14.13.4.7.1 A t test with the Bonferroni adjustment is used as an alternative to Dunnett's Procedure when, as in this set of data, the number of replicates is not the same for all concentrations. Like Dunnett's Procedure, it uses a pooled estimate of the variance, which is equal to the error value calculated in an analysis of variance. To obtain an estimate of the pooled variance, construct an ANOVA table as described in Table 25.

TABLE 25. ANOVA TABLE

Source	df	Sum of Squares (SS)	Mean Square (MS) (SS/df)
Between	p - 1	SSB	S_B^2 = SSB/(p-1)
Within	N - p	SSW	S_W^2 = SSW/(N-p)
Total	N - 1	SST	

Where: p = number of concentration levels including the control

N = total number of observations $n_1 + n_2 ... + n_p$

n_i = number of observations in concentration i

$$SSB = \sum_{i=1}^{p} T_i^2/n_i - G^2/N$$ Between Sum of Squares

$$SST = \sum_{i=1}^{p} \sum_{j-1}^{n_j} Y_{ij}^2 - G^2/N$$ Total Sum of Squares

$$SSW = SST - SSB$$ Within Sum of Squares

G = the grand total of all sample observations, $G = \sum_{i=1}^{p} T_i$

T_i = the total of the replicate measurements for concentration i

Y_{ij} = the jth observation for concentration i (represents the mean dry weight of the mysids for concentration i in test chamber j)

14.13.4.7.2 For the data in this example:

$n_1 = 8$ $n_2 = 7$ $n_3 = 7$

$N = 22$

$T_1 = Y_{11} + Y_{12} + ... + Y_{18} = 11.5$

$T_2 = Y_{21} + Y_{22} + ... + Y_{27} = 4.94$

$T_3 = Y_{31} + Y_{32} + ... + Y_{37} = 3.65$

$G = T_1 + T_2 + T_3 = 20.09$

$$SSB = \sum_{i=1}^{p} T_i^2/n_i - G^2/N$$

$$= \frac{132.25}{8} + \frac{24.40}{7} + \frac{13.32}{7} - \frac{403.61}{22} = 3.57$$

$$SST = \sum_{i=1}^{p} \sum_{j=1}^{n_j} Y_{ij}^2 - G^2/N$$

$$= 23.396 - \frac{403.61}{22} = 5.05$$

SSW = SST - SSB = 5.05 - 3.57 = 1.48

S_B^2 = SSB/(p-1) = 3.57/(3-1) = 1.785

S_W^2 = SSW/(N-p) = 1.48/(22-3) = 0.078

14.13.4.7.3 Summarize these calculations in the ANOVA table (Table 26).

TABLE 26. ANOVA TABLE FOR THE T TEST WITH BONFERRONI'S ADJUSTMENT EXAMPLE

Source	df	Sum of Squares (SS)	Mean Square (MS) (SS/df)
Between	2	3.57	1.785
Within	19	1.48	0.078
Total	21	5.05	

14.13.4.7.4 To perform the individual comparisons, calculate the t statistic for each concentration, and control combination as follows:

$$t_i = \frac{(\bar{Y}_1 - \bar{Y}_i)}{S_w\sqrt{(1/n_1) + (1/n_i)}}$$

Where: $\bar{Y}_i$ = mean proportion of females with eggs for concentration i

$\bar{Y}_1$ = mean proportion of females with eggs for the control

S_W = square root of the within mean square

n_1 = number of replicates for the control

n_i = number of replicates for concentration i

14.13.4.7.5 Table 27 includes the calculated t values for each concentration and control combination. In this example, comparing the 50.0 ppb concentration with the control the calculation is as follows:

$$t_2 = \frac{(1.44 - 0.52)}{[0.279\sqrt{(1/8) - (1/7)}]}$$

$$= 5.05$$

TABLE 27. CALCULATED T VALUES

Test Concentration (ppb)	i	t_i
50.0	2	5.05
100.0	3	6.37

14.13.4.7.6 Since the purpose of this test is to detect a significant reduction in mean proportion of females with eggs, a one-sided test is appropriate. The critical value for this one-sided test is found in Table 5, Appendix D, Critical Values for the t test with Bonferroni's adjustment. For an overall alpha level of 0.05, 19 degrees of freedom for error and two concentrations (excluding the control) the approximate critical value is 2.094. The mean proportion for concentration "i" is considered significantly less than the mean proportion for the control if t_i is greater than the critical value. Therefore, the 50.0 ppb and the 100.0 ppb concentrations have significantly lower mean proportion of females with eggs than the control. Hence the LOEC for fecundity is 50.0 ppb.

14.13.4.7.7 To quantify the sensitivity of the test, the minimum significant difference (MSD) that can be detected statistically may be calculated.

$$MSD = t\ S_w\sqrt{(1/n_1) + (1/n)}$$

Where: t = the critical value for the t test with Bonferroni's adjustment

S_W = the square root of the within mean square

n = the common number of replicates at each concentration (this assumes equal replication at each concentration)

n_1 = the number of replicates in the control

14.13.4.7.8 In this example:

$$MSD = 2.094\,(0.279)\sqrt{(1/8) + (1/7)}$$

$$= 2.094\ (0.279)(0.518)$$

$$= 0.303$$

14.13.4.7.9 Therefore, for this set of data, the minimum difference that can be detected as statistically significant is 0.30.

14.13.4.7.10 The MSD (0.30) is in transformed units. To determine the MSD in terms of percent of females with eggs, carry out the following conversion.

1. Subtract the MSD from the transformed control mean.

$$1.44 - 0.30 = 1.14$$

2. Obtain the untransformed values for the control mean and the difference calculated in 4.10.1.

$$[\ \text{Sine}\ (1.44)\ ]^2 = 0.983$$

$$[\ \text{Sine}\ (1.14)\ ]^2 = 0.823$$

3. The untransformed MSD (MSD_u) is determined by subtracting the untransformed values from 14.13.4.7.10.2.

$$MSD_u = 0.983 - 0.823 = 0.16$$

14.13.4.7.11 Therefore, for this set of data, the minimum difference in mean proportion of females with eggs between the control and any copper concentration that can be detected as statistically significant is 0.16.

14.13.4.7.12 This represents a 17% decrease in proportion of females with eggs from the control.

14.13.4.8 Calculation of the ICp

14.13.4.8.1 The fecundity data in Table 4 are utilized in this example. Table 28 contains the mean proportion of females with eggs for each toxicant concentration. As can be seen, the observed means are monotonically nonincreasing with respect to concentration. Therefore, it is not necessary to smooth the means prior to calculating the IC. Figure 20 gives a plot of the response curve.

TABLE 28. MYSID, *MYSIDOPSIS BAHIA*, MEAN MEAN PROPORTION OF FEMALES WITH EGGS

Toxicant Conc. (ppb)	i	Response Means Y^i (mg)	Smoothed Mean M_i (mg)
Control	1	0.934	0.934
50.0	2	0.426	0.426
100.0	3	0.317	0.317
210.0	4	0.000	0.000
450.0	5	0.010	0.000

14.13.4.8.2 An IC25 and IC50 can be estimated using the Linear Interpolation Method. A 25% reduction in mean proportion of females with eggs, compared to the controls, would result in a mean proportion of 0.701, where $M_1(1-p/100) = 0.934(1-25/100)$. A 50% reduction in mean proportion of females with eggs, compared to the could would result in a mean proportion of 0.467. Examining the means and their associated concentrations (Table 28), the response, 0.701, is bracketed by $C_1 = 0$ ppb and $C_2 = 50$ ppb. The response, 0.467, is bracketed by $C_1 = 0$ ppb and $C_2 = 50$ ppb.

14.13.4.8.3 Using the equation in Section 4.2 from Appendix L, the estimate of the IC25 is calculated as follows:

$$ICp = C_j + [M_1(1 - p/100) - M_j]\ \frac{(C_{(j+1)} - C_j)}{(M_{(j+1)} - M_j)}$$

$$IC25 = 0 + [0.934(1 - 25/100) - 0.934] \quad \frac{(50 - 0)}{(0.426 - 0.934)}$$

$$= 23 \text{ ppb.}$$

14.13.4.8.4 Using the equation in Section 4.2 from Appendix L, the estimate of the IC50 is calculated as follows:

$$ICp = C_j + [M_1 \ (1 - p/100) - M_j] \frac{C_{(j+1)} - C_j)}{(M_{(j+1)} - M_j)}$$

$$IC50 = 0 + [0.934(1 - 50/100) - 0.934] \quad \frac{(50 - 0)}{(0.426 - 0.934)}$$

$$= 46 \text{ ppb.}$$

14.13.4.8.5 When the ICPIN program was used to analyze this set of data, requesting 80 resamples, the estimate of the IC25 was 29.9745 ppb. The empirical 95.0% confidence interval for the true mean was 20.0499 ppb to 30.5675 ppb. The computer program output for the IC25 for this data set is shown in Figure 21. This value is extrapolated below the lowest test concentration and data should be used cautiously.

14.13.4.8.6 When the ICPIN program was used to analyze this set of data for the IC50, requesting 80 resamples, the estimate of the IC50 was 45.9490 ppb. The empirical 95.0% confidence interval for the true mean was 40.1467 ppb to 63.0931 ppb. The computer program output for the IC50 for this data set is shown in Figure 22.

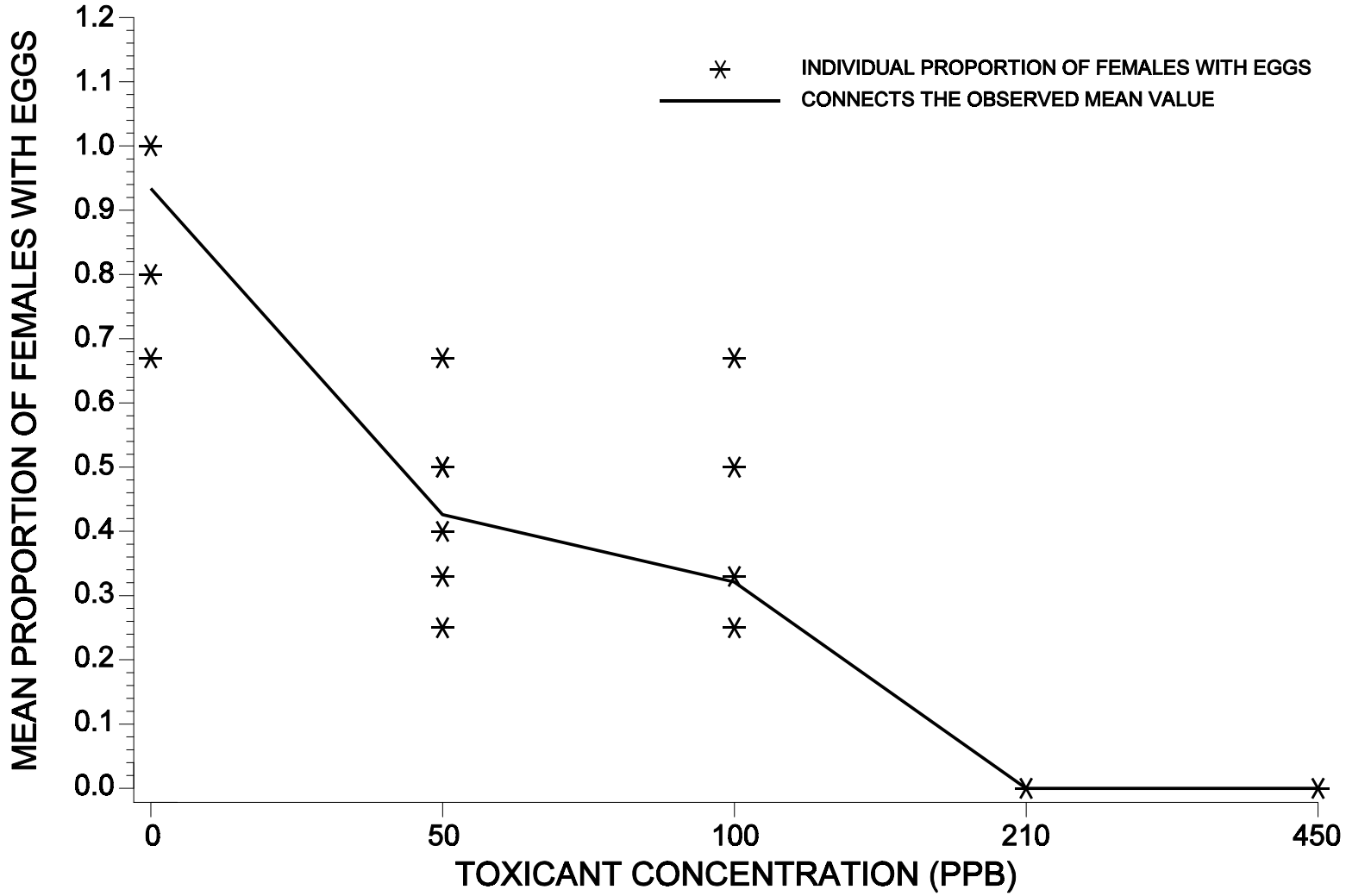

Figure 20. Plot of the mean proportion of female mysids, *Mysidopsis bahia*, with eggs

Conc. ID	1	2	3	4
Conc. Tested	0	50	100	210
Response 1	1	.5	.3	0
Response 2	1	.33	.5	0
Response 3	.67	.67	0	0
Response 4	1	.4	.5	0
Response 5	1	.5	.67	0
Response 6	.8	.25	0	0
Response 7	1	.33	.25	0
Response 8	1			0

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Effluent
Test Start Date: Test Ending Date:
Test Species: MYSID SHRIMP, Mysidopsis bahia
Test Duration: fecundity
DATA FILE: mysidfe.icp
OUTPUT FILE: mysidfe.i25

Conc. ID	Number Replicates	Concentration µg/l	Response Means	Standard. Dev.	Pooled Response Means
1 8	0.000	0.934	0.127	0.934	
2 7	50.000	0.426	0.142	0.426	
3 7	100.000	0.317	0.257	0.317	
4 8	210.000	0.000	0.000	0.000	

The Linear Interpolation Estimate: 29.9745 Entered P Value: 25

Number of Resamplings: 80
The Bootstrap Estimates Mean: 23.8871 Standard Deviation: 3.0663
Original Confidence Limits: Lower: 20.0499 Upper: 30.5765
Resampling time in Seconds: 1.37 Random Seed: 1918482350

Figure 21. ICPIN program output for the IC25.

Conc. ID	1	2	3	4
Conc. Tested	0	50	100	210
Response 1	1	.5	.3	0
Response 2	1	.33	.5	0
Response 3	.67	.67	0	0
Response 4	1	.4	.5	0
Response 5	1	.5	.67	0
Response 6	.8	.25	0	0
Response 7	1	.33	.25	0
Response 8	1			0

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Effluent
Test Start Date: Test Ending Date:
Test Species: MYSID SHRIMP
Test Duration: fecundity
DATA FILE: mysidfe.icp
OUTPUT FILE: mysidfe.i50

-Conc. ID	Number Replicates	Concentration µg/l	Response Means	Std. Dev.	Pooled Response Means
1 8	0.000	0.934	0.127	0.934	
2 7	50.000	0.426	0.142	0.426	
3 7	100.000	0.317	0.257	0.317	
4 8	210.000	0.000	0.000	0.000	

The Linear Interpolation Estimate: 45.9490 Entered P Value: 50

Number of Resamplings: 80
The Bootstrap Estimates Mean: 47.8720 Standard Deviation: 8.2908
Original Confidence Limits: Lower: 40.1467 Upper: 63.0931
Resampling time in Seconds: 1.32 Random Seed: -391064242

Figure 22. ICPIN program output for the IC50.

14.14 PRECISION AND ACCURACY

14.14.1 PRECISION – Data on single-laboratory and multilaboratory precision are described below (Subsections 14.14.1.1 and 14.14.1.2). Single-laboratory precision is a measure of the reproducibility of test results when tests are conducted using a specific method under reasonably constant conditions in the same laboratory. Single-laboratory precision is synonymous with the terms within-laboratory precision and intralaboratory precision. Multilaboratory precision is a measure of the reproducibility of test results from different laboratories using the same test method and analyzing the same test material. Multilaboratory precision is synonymous with the term interlaboratory precision. Interlaboratory precision, as used in this document, includes both within-laboratory and between-laboratory components of variability. In recent multilaboratory studies, these two components of interlaboratory precision have been displayed separately (termed within-laboratory and between-laboratory variability) and combined (termed total interlaboratory variability). The total interlaboratory variability that is reported from these studies is synonymous with interlaboratory variability reported from other studies where individual variability components are not separated.

14.14.1.1 Single-Laboratory Precision

14.14.1.1.1 Data on the single-laboratory precision of the mysid survival, growth, and fecundity using copper (Cu) sulfate and sodium dodecyl sulfate (SDS) in natural seawater and in artificial seawater (GP2) are shown in Tables 29-33. In Tables 29-30 the coefficient of variation for the IC25, ranges from 18.0 to 35.0 and the IC50, ranges from 5.8 to 47.8, indicating acceptable test precision. Data in Tables 31-33 show no detectable differences between tests conducted in natural or artificial seawaters.

14.14.1.1.2 EPA evaluated within-laboratory precision of the Mysid, *Mysidopsis bahia*, Survival, Growth, and Fecundity Test using a database of routine reference toxicant test results from 10 laboratories (USEPA, 2000b). The database consisted of 130 reference toxicant tests conducted in 10 laboratories using a variety of reference toxicants including: chromium, copper, and potassium chloride. Among the 10 laboratories, the median within-laboratory CV calculated for routine reference toxicant tests was 28% for the IC25 growth endpoint. In 25% of laboratories, the within-laboratory CV was less than 24%; and in 75% of laboratories, the within-laboratory CV was less than 32%.

14.14.1.2 Multilaboratory Precision

14.14.1.2.1 In 2000, EPA conducted an interlaboratory variability study of the Mysid, *Mysidopsis bahia*, Survival, Growth, and Fecundity Test (USEPA, 2001a; USEPA, 2001b). In this study, each of 11 participant laboratories tested 4 blind test samples that included some combination of blank, effluent, reference toxicant, and receiving water sample types. The blank sample consisted of bioassay-grade FORTY FATHOMS® synthetic seawater, the effluent sample was a municipal wastewater spiked with KCl, the receiving water sample was a natural seawater spiked with KCl, and the reference toxicant sample consisted of bioassay-grade FORTY FATHOMS® synthetic seawater spiked with KCl. Of the 44 *Mysidopsis bahia* Survival, Growth, and Fecundity tests conducted in this study, 97.7% were successfully completed and met the required test acceptability criteria. Of seven tests that were conducted on blank samples, none showed false positive results for survival, growth, or fecundity endpoints. Results from the reference toxicant, effluent, and receiving water sample types were used to calculate the precision of the method. Table 34 shows the precision of the IC25 for each of these sample types. Averaged across sample types, the total interlaboratory variability (expressed as a CV%) was 41.3% for growth IC25 results. Table 35 shows the frequency distribution of survival and growth NOEC endpoints for each sample type. For the survival endpoint, NOEC values spanned three concentrations for the reference toxicant, effluent, and receiving water sample types. The percentage of values within one concentration of the median was 100% for each of the sample types. For the growth endpoint, NOEC values spanned four concentrations for the reference toxicant sample type and three concentrations for the effluent and receiving water sample types. The percentage of values within one concentration of the median was 92.3%, 100%, and 100% for the reference toxicant, effluent, and receiving water sample types, respectively. For the fecundity endpoint, NOEC values spanned three concentrations for the reference toxicant, the effluent, and the receiving water sample types. The percentage of values within one concentration of the median was 75.0%, 87.5%, and 66.7% for the reference toxicant, effluent, and receiving water sample types, respectively.

14.14.2 ACCURACY

14.14.2.1 The accuracy of toxicity tests cannot be determined.

TABLE 29. SINGLE-LABORATORY PRECISION OF THE MYSID, *MYSIDOPSIS BAHIA*, SURVIVAL, GROWTH, AND FECUNDITY TEST PERFORMED IN NATURAL SEAWATER, USING JUVENILES FROM MYSIDS CULTURED AND SPAWNED IN NATURAL SEAWATER, AND COPPER (Cu) SULFATE AS A REFERENCE TOXICANT[1,2,3,4,5,6]

Test Number	NOEC (µg/L)	IC25 (µg/L)	IC50 (µg/L)	Most Sensitive Endpoint[7]
1	63	96.1	NC[8]	S
2	125	138.3	175.5	S
3	125	156.3	187.5	S
4	125	143.0	179.9	S
5	125	157.7	200.3	S
n:	5	5	4	
Mean:	NA	138.3	185.8	
CV(%):	NA	18.0	5.8	

1 Data from USEPA (1988a) and USEPA (1991a).
2 Tests performed by Randy Cameleo, ERL-N, USEPA, Narragansett, RI.
3 Eight replicate exposure chambers, each with five juveniles, were used for the control and each toxicant concentration. The temperature of the test solutions was maintained at 26 ± 1°C.
4 Copper concentrations in Tests 1-2 were: 8, 16, 31, 63, and 125 mg/L. Copper concentrations in Tests 3-6 were, 16, 31, 63, 125, and 250 µg/L.
5 NOEC Range: 63 - 125 µg/L (this represents a difference of two exposure concentrations).
6 For a discussion of the precision of data from chronic toxicity tests see Section 4, Quality Assurance.
7 Endpoints: G=Growth; S=Survival.
8 NC = No linear interpolation estimate could be calculated from the data, since none of the group response means were less than 50 percent of the control concentrations.

TABLE 30. SINGLE-LABORATORY PRECISION OF THE MYSID, *MYSIDOPSIS BAHIA*, SURVIVAL, GROWTH, AND FECUNDITY TEST PERFORMED IN NATURAL SEAWATER, USING JUVENILES FROM MYSIDS CULTURED AND SPAWNED IN NATURAL SEAWATER, AND SODIUM DODECYL SULFATE (SDS) AS A REFERENCE TOXICANT[1,2,3,4,5,6]

Test Number	NOEC (mg/L)	IC25 (mg/L)	IC50 (mg/L)	Most Sensitive Endpoint[7]
1	2.5	4.5	NC[9]	S
2	< 0.3	NC[8]	NC[9]	S
3	< 0.6	NC[8]	NC[9]	S
4	5.0	7.8	NC[9]	S
5	2.5	3.6	4.6	S
6	5.0	7.0	9.3	S
n:	4	4	2	
Mean:	NA	5.7	6.9	
CV(%):	NA	35.0	47.8	

1 Data from USEPA (1988a) and USEPA (1991a).
2 Tests performed by Randy Cameleo, ERL-N, USEPA, Narragansett, RI.
3 Eight replicate exposure chambers, each with five juveniles, were used for the control and each toxicant concentration. The temperature of the test solutions was maintained at 26 ± 1°C.
4 SDS concentrations in Tests 1-2 were: 0.3, 0.6, 1.3, 2.5, and 5.0 mg/L. SDS concentrations in Tests 3-4 were: 0.6, 1.3, 2.5, 5.0 and 10.0 mg/L. SDS concentrations in Tests 5-6 were: 1.3, 2.5, 5.0, 10.0, and 20.0 mg/L.
5 NOEC Range: < 0.3 - 5.0 mg/L (this represents a difference of four exposure concentrations).
6 For a discussion of the precision of data from chronic toxicity tests see Section 4, Quality Assurance.
7 Endpoints: G=Growth; S=Survival.
8 NC = No linear interpolation estimate could be calculated from the data, since none of the group response means were less than 75 percent of the control response mean.
9 NC = No linear interpolation estimate could be calculated from the data, since none of the group response means were less than 50 percent of the control response mean.

TABLE 31. COMPARISON OF SURVIVAL (LC50)[1], GROWTH AND FECUNDITY (IC50)[1] RESULTS FROM 7-DAY TESTS WITH THE MYSID, *MYSIDOPSIS BAHIA*, USING NATURAL SEAWATER (NSW) AND ARTIFICIAL SEAWATER (GP2) AS DILUTION WATER AND SODIUM DODECYL SULFATE (SDS) AS A REFERENCE TOXICANT

	Survival LC50		Growth IC50		Fecundity IC50	
Test	NSW	GP2	NSW	GP2	NSW	GP2
1	16.2	16.3	16.8	16.3	12.0	10.9
2	20.5	19.2	24.2	23.3	20.1	18.5
3	--[2]	21.9	--[2]	24.4	--[2]	21.7

[1] All LC50/IC50 values in mg/L.
[2] No test performed.

TABLE 32. COMPARISON OF SURVIVAL (LC50)[1], GROWTH AND FECUNDITY (IC50)[1] RESULTS FROM 7-DAY TESTS WITH THE MYSID, *MYSIDOPSIS BAHIA*, USING NATURAL SEAWATER (NSW) AND ARTIFICIAL SEAWATER (GP2) AS DILUTION WATER AND COPPER (Cu) SULFATE AS A REFERENCE TOXICANT

	Survival LC50		Growth IC50		Fecundity IC50	
Test	NSW	GP2	NSW	GP2	NSW	GP2
1	177	182	208	186	177	125
2	--[2]	173	--[2]	210	--[2]	142
3	190	174	195	179	168	186

[1] All LC50/IC50 values in µg/L.
[2] No test performed.

TABLE 33. CONTROL RESULTS FROM 7-DAY SURVIVAL, GROWTH, AND FECUNDITY TESTS WITH THE MYSID, *MYSIDOPSIS BAHIA*, USING NATURAL SEAWATER AND ARTIFICIAL SEAWATER (GP2) AS A DILUTION WATER

	Control [1]					
	Survival (%)		Growth (mg)		Fecundity (%)	
Test	NSW	GP2	NSW	GP2	NSW	GP2
1	98	93	0.32	0.32	73	77
2	80	90	0.40	0.43	100	95
3	--[2]	95	--[2]	0.40	--[2]	100
4	94	84	0.34	0.37	89	83
5	--[2]	94	--[2]	0.36	--[2]	83
6	80	75	0.40	0.41	79	93

[1] Survival as percent of mysids alive after 7 days; growth as mean individual dry weight; fecundity as percent females with eggs.
[2] No test performed.

TABLE 34. PRECISION OF POINT ESTIMATES FOR VARIOUS SAMPLE TYPES[1]

Test Endpoint	Sample Type	CV (%)[2]		
		Within-lab[3]	Between-lab[4]	Total[5]
IC25 for Growth	Reference toxicant	8.69	40.0	40.9
	Effluent	5.26	36.6	37.0
	Receiving water	-	-	45.9
	Average	6.98	38.3	41.3

[1] From EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b).

[2] CVs were calculated based on the within-laboratory component of variability, the between-laboratory component of variability, and the total interlaboratory variability (including both within-laboratory and between-laboratory components). For the receiving water sample type, within-laboratory and between-laboratory components of variability could not be calculated since the study design did not provide within-laboratory replication for this sample type.

[3] The within-laboratory (intralaboratory) component of variability for duplicate samples tested at the same time in the same laboratory.

[4] The between-laboratory component of variability for duplicate samples tested at different laboratories.

[5] The total interlaboratory variability, including within-laboratory and between-laboratory components of variability. The total interlaboratory variability is synonymous with interlaboratory variability reported from other studies where individual variability components are not separated.

TABLE 35. FREQUENCY DISTRIBUTION OF HYPOTHESIS TESTING RESULTS FOR VARIOUS SAMPLE TYPES[1]

Test Endpoint	Sample Type	Median NOEC Value	% of Results at the Median	% of Results ±1[2]	% of Results ≥2[3]
Survival NOEC	Reference toxicant	25%	53.8	46.2	0.00
	Effluent	12.5%	46.7	53.3	0.00
	Receiving water	12.5%	37.5	62.5	0.00
Growth NOEC	Reference toxicant	25%	53.8	38.5	7.69
	Effluent	12.5%	46.7	53.3	0.00
	Receiving water	12.5%	50.0	50.0	0.00
Fecundity NOEC	Reference toxicant	18.8%	-[4]	75.0	25.0
	Effluent	25%	62.5	25.0	12.5
	Receiving water	9.38%	-[4]	66.7	33.3

[1] From EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b).
[2] Percent of values at one concentration interval above or below the median. Adding this percentage to the percent of values at the median yields the percent of values within one concentration interval of the median.
[3] Percent of values two or more concentration intervals above or below the median.
[4] The median NOEC fell between test concentrations, so no test results fell precisely on the median.